What is claimed is:

1. A method for determining whether a HIV-1 has an increased likelihood of being hypersusceptible to treatment with a protease inhibitor, comprising: detecting whether the protease encoded by said HIV exhibits the presence or absence of a mutation associated with hypersusceptibility to treatment with said protease inhibitor at amino acid position 16, 20, 33, 36, 37, 39, 45, 65, 69, 77, 89 or 93 of an amino acid sequence of said protease, wherein the presence of said mutation indicates that the HIV has an increased likelihood of being hypersusceptible to treatment with the protease inhibitor, with the proviso that said mutation is not L33F.

2. The method of claim 1, wherein the protease has a sequence that is greater than 80% identical to SEQ ID NO:1.

3. A method for determining whether an individual infected with HIV-1 has an increased likelihood of being hypersusceptible to treatment with a protease inhibitor, comprising detecting, in a sample from said individual, the presence or absence of a mutation associated with hypersusceptibility to treatment with said protease inhibitor at amino acid position 16, 20, 33, 36, 37, 39, 45, 65, 69, 77, 89 or 93 of the amino acid sequence of the protease of the HIV-1, wherein the presence of said mutation indicates that the individual has an increased likelihood of being hypersusceptible to treatment with the protease inhibitor, with the proviso that said mutation is not L33F.

4. The method of claim 3, wherein the protease has a sequence that is greater than 80% identical to SEQ ID NO:1.

5. An isolated oligonucleotide between about 10 and about 40 nucleotides long encoding a portion of an HIV protease that comprises a mutation at amino acid position 16, 20, 33, 36, 37, 39, 45, 65, 69, 77, 89 or 93 of an amino acid sequence of said protease in said human immunodeficiency virus, wherein the mutation is associated with hypersusceptibility to a protease inhibitor, with the proviso that said mutation is not L33F.

6. The method of claim 1, wherein said protease inhibitor is amprenavir.

7. The method of claim 6, wherein said amino acid position is 20, 36, 39, 65, 69, 77 or 89.

8. The method of claim 1, wherein said protease inhibitor is indinavir.

9. The method of claim 8, wherein said amino acid position is 16, 39 or 65.

10. The method of claim 1, wherein said protease inhibitor is nelfinavir.

11. The method of claim 10, wherein said amino acid position is 16, 39, 65, 69 or 89.

12. The method of claim 1, wherein said protease inhibitor is ritonavir.

13. The method of claim 12, wherein said amino acid position is 39, 65 or 93.

14. The method of claim 1, wherein said protease inhibitor is saquinavir.

15. The method of claim 14, wherein said amino acid position is 33, 37, 45, 65 or 77, with the proviso that the mutation at amino acid position 33 is not 33F.

16. The method of claim 1, wherein said protease inhibitor is lopinavir.

17. The method of claim 16, wherein said amino acid position is 33, 39, 65, 77 or 93, with the proviso that the mutation at amino acid position 33 is not 33F.

102(b) 18. The method of claim 3, wherein the individual is undergoing or has undergone prior treatment with an anti-viral drug.

102(b) 19. The method of claim 1, wherein the method comprises detecting the presence or absence of a mutation associated with hypersusceptibility to treatment with said protease inhibitor at at least 2, 3, 4, 5, 6, 7, 8, 9, 10, 11 or 12 of the amino acid positions.

20. A method for determining whether a HIV-1 has a decreased likelihood of being hypersusceptible to a protease inhibitor, comprising: detecting whether the protease encoded by said HIV-1 exhibits the presence or absence of a mutation negatively associated with hypersusceptibility to said protease inhibitor at amino acid position 10, 15, 36, 41, 57, 60, 63, 71 or 93 of an amino acid sequence of said protease, wherein the presence of said mutation indicates that the HIV has a decreased likelihood of being hypersusceptible to the protease inhibitor.

21. A method for determining whether an individual infected with HIV-1 has a decreased likelihood of being hypersusceptible to treatment with a protease inhibitor, comprising detecting, in a sample from said individual, the presence or absence of a mutation negatively associated with hypersusceptibility to treatment with said protease inhibitor at amino acid position 10, 15, 36, 41, 57, 60, 63, 71 or

93 of the amino acid sequence of the protease of the HIV-1, wherein the presence of said mutation indicates that the individual has a decreased likelihood of being hypersusceptible to treatment with the protease inhibitor.

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     FILE 'USPATFULL' ENTERED AT 13:01:39 ON 23 JUL 2007
               E PARKIN NEIL T/IN
L1           27 S E3
L2           12 S L1 AND (PR/CLM OR PROTEASE/CLM)
L3           11 S L2 AND (HIV?/CLM OR HUMAN IMMUNODEFICIENCY VIRUS/CLM)
L4            3 S L3 AND (HYPERSUSCEPTIBIL?/CLM OR INCREASE? SUSCEPTIBILIT?/CLM
L5         8918 S (HIV?/CLM OR HUMAN IMMUNODEFICIENCY VIRUS/CLM)
L6         1383 S L5 AND (PR/CLM OR PROTEASE/CLM)
L7            3 S L6 AND (HYPERSUSCEPTIB?/CLM OR INCREASE? SUSCEPTIBIL?/CLM)
L8            0 S L7 NOT L4

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L9           27 S E3
L10          17 S L9 AND (HIV OR HUMAN IMMUNODEFICIENCY VIRUS)
L11          12 S L10 AND (PR OR PROTEASE)
L12           3 S L11 AND (HYPERSUSCEPTIB? OR INCREAS? SUSCEPTIB?)
L13       25650 S (HIV OR HUMAN IMMUNODEFICIENCY VIRUS)
L14        2534 S L13 AND (PR OR PROTEASE)
L15           3 S L14 AND (HYPERSUSCEPTIB? OR INCREASE? SUSCEPTIB?)
L16           0 S L15 NOT L12

     FILE 'MEDLINE' ENTERED AT 13:09:37 ON 23 JUL 2007
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L17          51 S E3-E5
L18          27 S L17 AND (PR OR PROTEASE)
L19          22 S L18 AND (PR/AB OR PROTEASE/AB)
L20          21 S L19 AND (HIV?/AB OR HUMAN IMMUNODEFICIENCY VIRUS/AB)
L21           6 S L20 AND (HYPERSUSCEPTIB?/AB OR INCREASED SUSCEPTIB?/AB)
L22      122819 S (HIV?/AB OR HUMAN IMMUNODEFICIENCY VIRUS/AB)
L23        7120 S L22 AND (PR/AB OR PROTEASE/AB)
L24          33 S L23 AND (HYPERSUSCEPTI?/AB OR INCREASED SUSCEPTIBIL?/AB)
L25          27 S L24 NOT L21
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EDITORIAL COMMENT

Genotype–phenotype discordance: the evolution in our understanding HIV-1 drug resistance

Andrew R. Zolopa

AIDS 2003, **17**:1077–1078

Keywords: HIV, protease, genotype, phenotype, resistance, lopinavir, amprenavir

Although HIV resistance testing is now considered standard of care in resource-rich countries, both genotype and phenotype tests have limitations [1]. In this issue of *AIDS*, Parkin and colleagues from Virologic Inc. illustrate an important limitation of current genotype interpretation algorithms for antiretroviral agents such as lopinavir/ritonavir (LPV/r; Kaletra), which require multiple mutations for clinically significant resistance [2]. The algorithms for such drugs are complex both in terms of the number of mutations that contribute to resistance and in the various patterns of mutations that result in a virus with clinically significant resistance. Attempting to put this complexity into a simple set of rules is an ongoing challenge. Phenotype tests, however, appear somewhat less 'sensitive' than genotyping to what could be seen as evolving resistance. Faced with the limitations of resistance tests, many clinicians order both tests in an attempt to make optimal treatment decisions for their patients. Not infrequently, however, clinicians who obtain both a phenotype and genotype test find the results to be in disagreement, or 'discordant', that is, one test result is interpreted as drug sensitive while the other is interpreted as drug resistant. Understanding the reasons for these discordant results should enable the clinician to make better use of these tests and thereby make better treatment decisions.

Some of the discordance between genotype and phenotype is simply a reflection of our incomplete and still evolving understanding of drug resistance. This discordance appears to be a consequence of incorrect interpretation of the test results, that is to say it is not the results that are in disagreement it is the interpretation. After all, a simple count of mutations or a single phenotype fold change (FC) in the 50% inhibitory concentration value used as a barrier to separate 'sensitive' from 'resistant' virus is somewhat arbitrarily drawn. These arbitrary separations lead to misclassification and apparent discordances. Apparent discordant results are illustrated in Parkin *et al.* [2] by those viruses classified by the original LPV/r scoring system as genotypically 'sensitive' (i.e., less than six mutations) but phenotypically 'resistant' (i.e., FC > 10) [3]. Parkin *et al.* developed a new genotypic interpretation algorithm that better predicts LPV/r phenotypic susceptibility. By looking closely at the results in which there was genotypic–phenotypic discordance (sensitive by genotyping but reduced susceptibility by phenotyping), the authors were able to improve our understanding of the LPV/r genotypic resistance profile and eliminate a significant amount of the discordance.

There are several examples of what appears to be genotype–phenotype discordance of actual test results [4]. In these examples, the phenotype appears sensitive (i.e., not different from wild type) while the genotype reveals mutations generally associated with drug resistance. As demonstrated by Parkin *et al.* [2], mixtures of drug-resistant mutants and wild-type virus may not

See also p. 955

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always be detected by a shift in phenotype FC. It will depend on the specific mutation, the drug and the relative proportion of mutant/wild-type virus in the specimen. A second example involves transitional mutations. In cohorts of recently tested patients, investigators at the Centers for Disease Control and Prevention have shown that resistant viruses can back-mutate to intermediate or transitional forms that, by themselves, do not effect the phenotype and yet quickly re-emerge as resistant mutations with appropriate drug exposure [5]. Finally, antagonistic interactions between mutations in effect cancel each other's impact on the phenotype and result in a phenotype that appears 'sensitive'. These interactions have been demonstrated to occur within the nucleoside reverse transcriptase inhibitor class and between the nucleoside and non-nucleoside reverse transcriptase inhibitor classes [6,7].

Whatever the source of discordance, the question for the clinician is how best to incorporate this kind of information into a treatment plan. Many practitioners rely on the interpretation that accompanies the results of a genotype or phenotype test. Under this circumstance, having more information about the patient's resistance profile will not necessarily lead to better outcomes if the results are discordant and the clinician is left confused. The clinician must be an informed and critical consumer of this technology and the interpretation that accompanies the results. If the test results are discordant, the clinician must ask why the results appear to be at odds and then base a treatment plan on this more informed view.

Parkin and colleagues have furthered our understanding of the ever increasing complexity of genotypic resistance patterns to LPV/r and the potential for at least some degree of cross-resistance with amprenavir [2]. For clinicians trying to chose between the various 'boosted' protease inhibitor antiretroviral regimens, a phenotype test will likely be most helpful in summarizing this genotypic complexity into a result that can be used to optimize the regimen. However, an accompanying genotype analysis can help to inform the phenotype result by identifying mixtures, transitional mutations and antagonistic mutational interactions. Clinicians should consider consulting an expert in HIV drug resistance if the results of a resistance test(s) are confusing or discordant. Ultimately, the discordant results need to be correlated with virological response to specific treatment regimens before we can fully realize the benefits of these evolving technologies.

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Loss of antiretroviral drug susceptibility at low viral load during early virological failure in treatment-experienced patients

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Background: Clinical studies have demonstrated a correlation between the response to second-line antiretroviral therapy and the number of drugs in the regimen to which the virus is susceptible. These studies have largely been performed in patients with viral loads over 1000 copies/ml.

Objectives: To examine the evolution of resistance during early virological failure, and the potential role of susceptibility testing in patients with low viral loads (below 1000 copies/ml), in treatment-experienced patients.

Methods: Drug susceptibility and genotypes of HIV-1 from indinavir-experienced patients undergoing therapy with nelfinavir, saquinavir, abacavir and either a second nucleoside reverse transcriptase inhibitor (NRTI) or nevirapine were determined.

Results: Sixteen subjects were studied. Five of the ten subjects treated with nevirapine, and one of six treated with a second NRTI, achieved and maintained plasma HIV RNA < 500 copies/ml. Virus from the treatment failures lost susceptibility to one or more treatment drugs, including nelfinavir and/or saquinavir, after 4 to 36 weeks of treatment. In six of the ten failures, virus with new reductions in drug susceptibility was detected prior to failure. In five of the six failures who had at least one plasma sample with a viral load between 50 and 1000 copies/ml, reductions in susceptibility to one or more treatment drugs were detected (viral load range: 260 to 630 copies/ml).

Conclusions: Drug resistance can be detected at viral loads below 1000 copies/ml which may be predictive of treatment failure. Failure of a second line regimen was typically associated with early evolution of resistance in HIV protease.

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Keywords: Antriretroviral therapy, combination therapy, HIV drug resistance, protease inhibitors, reverse transcriptase inhibitors, reverse transcriptase-polymerase chain reaction, viral load

Introduction

The goal of antiretroviral therapy is to reduce HIV-1-associated morbidity and mortality, by suppressing viral replication to the maximum degree possible [1]. Unfortunately, failure to achieve complete suppression is common in clinical practice [2]. Although the advent of highly active antiretroviral therapy (HAART) has led to dramatic decreases in HIV-related morbidity and mortality [3], the development of virus with reduced susceptibility to antiretroviral drugs is frequently observed after virological failure of HAART [4–7].

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Table 5. Protease and reverse transcriptase genotypes of virus from treatment failures.

Subject no.	Time	Resistance-associated mutations	
		Protease	Reverse transcriptase
1	baseline	L10I, K20I, M36I, I54V, L63P, A71V, V82T	D67N, T69D, K70R, M184V, T215F, K219Q
	4 weeks	L10I, K20I, M36I, I54V, L63P, A71V, V82T	D67N, T69D, K70R, M184V, T215F, K219Q
	5 weeks	L10I, K20I, M36I, I54V, L63P, A71V, V82T	D67N, T69D, K70R, **Y181Y/C**, M184M/V, T215F, K219Q, **F227F/L**
	11 weeks	L10I, K20I, M36I, I54V, L63P, A71V, V82T	D67N, T69D, K70R, **V106A**, T215F, K219Q, F227L
2	baseline	L63P, G73G/C, L90L/M	M41L, T69A/D/N, V118V/I, M184V, L210W, T215Y
	10 weeks	**L10L/I**, L63P, G73C, L90M	M41L, **A98G**, M184V, L210W, T215Y
	14 weeks	**L10L/I**, **M46M/L**, L63P, G73C, L90M	M41L, **A98G**, M184V, L210W, T215Y
	18 weeks	**L10L/I**, **M46M/L**, L63P, G73C, L90M	M41L, **A98G**, M184V, L210W, T215Y
	22 weeks	**L10L/I**, **M46M/L**, L63P, G73C, **V77V/I**, L90M	M41L, **A98G**, M184M/V, L210W, T215Y
6	baseline	L10I, M46I, L63P, A71V, L90M	D67G, K70R, M184V, K219Q
	5 weeks	L10I, M46I, L63P, A71V, L90M	D67G, K70R, M184V, **T215T/A**, K219Q
	9 weeks	L10I/V, **K20K/R**, **M36M/I**, M46M/I/L, L63P, A71V **V82V/A**, **I84I/V**, L90M	D67G, K70R, M184V, **T215T/A/I/V**, K219Q
	18 weeks	L10I/V, **K20R**, **M36M/I**, M46M/I/L, **G48G/V**, L63P, A71V, **V82V/A**, L90M	D67G, K70R, M184V, **T215T/A/I/V**, K219Q
7	baseline	L10L/I, L24I, M46I/L, I54A/V, L63P, A71L/V, V82A, L90L/M	L74V, M184V
	8 weeks	L10I, **G48G/V**, I54V, L63P, A71V, V82A, L90M	L74V, M184V
	16weeks	L10I, **G48V**, I54V, A71V, **V77V/I**, V82A, L90M	L74V, M184V
	36 weeks	L10I, **G48V**, I54V, A71V, **V77I**, V82A, L90M	L74V, M184V
10	baseline	K20I, L63P, V77I	M184V
	13 weeks	K20I, L63P, **A71A/T**, **G73G/S**, V77I, **L90M**	M184V
	17 weeks	K20K/I, L63P, **A71A/I/T/V**, **G73G/S**, V77I, **L90M**	M184V, **T215T/N/S/Y**
	26 weeks	K20I, L63P, **A71A/I/T/V**, **G73G/S**, V77I , **L90M**	M184V, **T215Y**
	33 weeks	K20I, L63P, **A71A/I/T/V**, **G73G/S**, V77I, **L90M**	M184V, **T215Y**
12	baseline	L10I, L24I, M46I, I54V, L63P, V82A	D67N, T69D, K70R, M184V, T215F, K219Q
	36 weeks	L10I, L24I, M46I, I54V, L63P, V82A	D67N, T69D, **Y181C**, M184M/V, T215F, K219Q
	48 weeks	L10I, L24I, M46I, I54V, L63P, V82A	D67N, T69D, K70K/R, **Y181C**, M184M/V, T215F, K219Q
14	baseline	L10I, M46I, L63L/P, G73A/T, V77I, L90M	M41L, L74I, M184V, L210W, T215Y
	4 weeks	L10I, M46I, L63L/P, G73S/T, V77I, L90M	M41L, **A62A/V**, L74I, **Y181Y/C**, M184V, L210W, T215Y, **M230M/L**
	8 weeks	L10I, M46I, L63P, G73S, V77I, L90M	M41L, L74I, **Y181C**, M184V, L210W, T215Y, **M230L**
	16 weeks	L10I, M46I, L63P, G73S/T, V77I, L90M	M41L, L74I, **Y181C**, M184V, L210W, T215Y, **M230L**
19	baseline	L10I, L24I, M46L, I54V, L63P, A71V, V82A	M41L, M184V, T215Y
	2 weeks	L10I, L24I, M46L, I54V, L63P, A71V, V82A	M41L, M184V, T215Y
	4 weeks	L10I, L24I, M46L, I54V, L63P, A71V, V82A	M41L, **L74V**, **K103N**, **Y181C**, M184V, T215Y
	8 weeks	L10I, L24I, **M36M/I**, M46L, I54V, L63P, A71V, V82A	M41L, **L74V**, **K103N**, **Y181C**, M184V, T215Y
	12 weeks	L10I, L24I, **M36M/I**, M46L, I54V, L63P, A71V, V82A	M41L, **L74V**, **K103N**, **Y181C**, M184V, T215Y
20	baseline	M46L, I54I/V, L63P, V82V/A, L90L/M	M41L, M184V, T215Y
	12 weeks	M46L, I54V, L63P, V82A, L90L/M	M41L, M184V, T215Y
	16 weeks	M46L, I54I/V, L63P, V82V/A, L90L/M	M41L, M184V, T215Y
	24 weeks	M46L, L63P, L90M	M41L, **D67N**, **K103N**, M184M/V, T215Y
	28 weeks	M46L, L63P, L90M	M41L, **D67N**, **K103N**, M184V, T215Y
	36 weeks	M46L, L63P, L90M	M41L, **D67N**, **K103N**, M184M/V, T215Y
21	baseline	L10I, M46M/I, I54I/V, L63Q, V82A	K103N, P225H
	12 weeks	L10I, **G48V**, L63Q, V82A	**D67D/G**, K103N, **M184M/I**, P225H
	16 weeks	L10L/I, **L24L/I/M**, M46M/I, **G48G/V**, L63Q, V82A	K103N, **M184M/I**, P225H
	20 weeks	L10I, **G48V**, L63Q, V82A	**D67D/G**, K103N, **M184I**, P225H
	24 weeks	L10I, **G48V**, L63Q, V82A	**D67D/G**, K103N, **M184I/V**, P225H

[a]Major resistance-associated mutations [23] are listed, as changes compared to the NL4-3 reference sequence. Mixtures are indicated by listing all possible amino acids at the codon in question separated by a slash.
Mutations in bold type represent changes from that patient's baseline genotype

TABLE 1

PROTEASE POSITIONS ASSOCIATED WITH HYPERSUSCEPTIBILITY

Protease Inhibitor	Positive Association	Negative Association
APV	20, 36, 39, 65, 69, 77, 89	10, 15
IDV	16, 39, 65	10, 57, 63, 93
NFV	16, 39, 65, 69, 89	10, 57, 63, 71
RTV	39, 65, 93	15, 57
SQV	33*, 37, 45, 65, 77	15, 36, 41, 57, 60
LPV	33*, 39, 65, 77, 93	none

* all mutations at position 33, except 33F
underlined positions were associated with the largest changes in mean FC

TABLE 2

Resistance-Associated Mutations

PROTEIN	AMINO ACID POSITIONS
PROTEASE	23, 24, 30, 32, 33F, 46, 47, 48, 50, 54, 82 (not I), 84, 88, 90
REVERSE TRANSCRIPTASE	41, 62, 65, 67, 69, 70, 74, 75, 77, 98G, 100, 101, 103, 106, 108, 115, 116, 151, 181, 184, 188, 190, 210, 215, 219, 225, 227, 236

TABLE 3

DISTRIBUTION OF FOLD CHANGE VALUES

FOLD CHANGE	APV	IDV	NFV	RTV	SQV	LPV
Mean	0.69	0.78	1.05	0.82	0.70	0.68
Median	0.71	0.78	1.05	0.81	0.71	0.69
90th Percentile	1.32	1.35	2.09	1.55	1.12	1.15
10th Percentile	0.35	0.44	0.54	0.45	0.44	0.40

TABLE 4

Summary of Regression Coefficients for Each Pair of Protease Inhibitors

	IDV	NFV	RTV	SQV	LPV
APV	0.64	0.58	0.70	0.48	0.71
IDV		0.79	0.68	0.62	0.71
NFV			0.71	0.49	0.58
RTV				0.60	0.77
SQV					0.72

"Susceptibility" refers to a virus' response to a particular drug. A virus that has decreased or reduced susceptibility to a drug has an increased resistance or decreased sensitivity to the drug. A virus that has increased or enhanced or greater susceptibility to a drug has an increased sensitivity or decreased resistance to the drug.

Phenotypic susceptibility of a virus to a given drug is a continuum. Nonetheless, it is practically useful to define a threshold or thresholds to simplify interpretation of a particular fold-change result. For drugs where sufficient clinical outcome data have been gathered, it is possible to define a "clinical cutoff value," as below.

"Hypersusceptibility" ("HS") refers to an enhanced or greater susceptibility to a drug, an increased sensitivity to a drug or decreased resistance to a drug. Hypersusceptibility is defined as a fold change ("FC") (see below) equal to or less than the 10^{th} percentile for each protease inhibitors' fold change distribution.

"Clinical Cutoff Value" refers to a specific point at which resistance begins and sensitivity ends. It is defined by the drug susceptibility level at which a patient's probability of treatment failure with a particular drug significantly increases. The cutoff value is different for different anti-viral agents, as determined in clinical studies. Clinical cutoff values are determined in clinical trials by evaluating resistance and outcomes data. Drug susceptibility (phenotypic) is measured at treatment initiation. Treatment response, such as change in viral load, is monitored at predetermined time points through the course of the treatment. The drug susceptibility is correlated with treatment response and the clinical cutoff value is determined by resistance levels associated with treatment failure (statistical analysis of overall trial results).

"IC_n" refers to Inhibitory Concentration. It is the concentration of drug in the patient's blood or *in vitro* needed to suppress the reproduction of a disease-causing microorganism (such as HIV) by n %. Thus, "IC_{50}" refers to the concentration of an anti-viral agent at which virus replication is inhibited by 50% of the level observed in the absence of the drug. "Patient IC_{50}" refers to the drug concentration required to inhibit replication of the virus from a patient by 50% and "reference IC_{50}" refers to the drug concentration required to inhibit replication of a reference or wild-type virus by 50%. Similarly, "IC_{90}" refers to the concentration of an anti-viral agent at which 90% of virus replication is inhibited.

A "fold change" is a numeric comparison of the drug susceptibility of a patient virus and a drug-sensitive reference virus. It is the ratio of the Patient IC_{50} to the drug-sensitive

reference IC_{50}, *i.e.*, Patient IC_{50}/Reference IC_{50} = Fold Change ("FC"). A fold change of 1.0 indicates that the patient virus exhibits the same degree of drug susceptibility as the drug-sensitive reference virus. A fold change less than 1 indicates the patient virus is more sensitive than the drug-sensitive reference virus. A fold change greater than 1 indicates the patient virus is less susceptible than the drug-sensitive reference virus. A fold change equal to or greater than the clinical cutoff value means the patient virus has a lower probability of response to that drug. A fold change less than the clinical cutoff value means the patient virus is sensitive to that drug.

A virus is "sensitive" to APV, IDV, NFV, SQV and RTV if it has an APV, IDV, NFV, SQV and RTV, respectively, fold change of less than 2.5. A virus is sensitive to LPV if it has an LPV fold change of less than 10.

A virus is "resistant" to APV, IDV, NFV, SQV and RTV if it has an APV, IDV, NFV, SQV and RTV, respectively, fold change of 2.5 or more. A virus is resistant to LPV if it has an LPV fold change of 10 or more.

A virus has an "increased likelihood of being hypersusceptible" to an anti-viral treatment if the virus has a property, for example, a mutation, that is correlated with hypersusceptibility to the anti-viral treatment. A property of a virus is correlated with hypersusceptibility if a population of viruses having the property is, on average, more susceptible to the anti-viral treatment than an otherwise similar population of viruses lacking the property. Thus, the correlation between the presence of the property and hypersusceptibility need not be absolute, nor is there a requirement that the property is necessary (*i.e.*, that the property plays a causal role in increasing susceptibility) or sufficient (*i.e.*, that the presence of the property alone is sufficient) for conferring hypersusceptibility.

A virus has an "decreased likelihood of being hypersusceptible" to an anti-viral treatment if there is a negative correlation which is statistically significant ($P < 0.05$) in at least one of the following statistical tests: the t-test for comparison of means, the non-parametric Kruskal-Wallis test or the Fisher's Exact test.

The term "% sequence homology" is used interchangeably herein with the terms "% homology," "% sequence identity" and "% identity" and refers to the level of amino acid sequence identity between two or more peptide sequences, when aligned using a sequence alignment program. For example, as used herein, 80% homology means the same thing as 80% sequence identity determined by a defined algorithm, and accordingly a homologue of a

or a prior viral strain from the same patient. Because the virus is directly exposed to each of the available anti-viral medications, results can be directly linked to treatment response. For example, if the patient virus shows resistance to a particular drug, that drug is avoided or omitted from the patient's treatment regimen, allowing the physician to design a treatment plan that is more likely to be effective for a longer period of time. Conversely, if the patient virus shows increased susceptibility to a particular drug, that drug can be repeated.

In another embodiment, the phenotypic analysis is performed using recombinant virus assays ("RVAs"). RVAs use virus stocks generated by homologous recombination between viral vectors and viral gene sequences, amplified from the patient virus. In some embodiments, the viral vector is a HIV vector and the viral gene sequences are protease and/or reverse transcriptase sequences.

In a preferred embodiment, the phenotypic analysis is performed using PHENOSENSE™ (ViroLogic Inc., South San Francisco, CA). *See* Petropoulos *et al.*, 2000, *Antimicrob. Agents Chemother.* 44:920-928; U.S. Patent Nos. 5,837,464 and 6,242,187. PHENOSENSE™ is a phenotypic assay that achieves the benefits of phenotypic testing and overcomes the drawbacks of previous assays. Because the assay has been automated, PHENOSENSE™ offers higher throughput under controlled conditions. The result is an assay that accurately defines the susceptibility profile of a patient's HIV isolates to all currently available antiretroviral drugs, and delivers results directly to the physician within about 10 to about 15 days of sample receipt. PHENOSENSE™ is accurate and can obtain results with only one round of viral replication, thereby avoiding selection of subpopulations of virus. The results are quantitative, measuring varying degrees of drug susceptibility, and sensitive – the test can be performed on blood specimens with a viral load of about 500 copies/mL and can detect minority populations of some drug-resistant virus at concentrations of 10% or less of total viral population. Furthermore, the results are reproducible and can vary by less than about 1.4-2.5 fold, depending on the drug, in about 95% of the assays performed.

PHENOSENSE™ can be used with nucleic acids from amplified viral gene sequences. As discussed in Section 5.4.1, the sample containing the virus may be a sample from a human or an animal infected with the virus or a sample from a culture of viral cells. In one embodiment, the viral sample comprises a genetically modified laboratory strain.

In some embodiments, the present invention provides a method for monitoring the effectiveness of an anti-viral treatment in an individual infected with a virus and undergoing or having undergone prior treatment with the same or different anti-viral treatment, comprising, detecting, in a sample of said individual, the presence or absence of an amino acid residue associated with hypersusceptibility to treatment the anti-viral treatment, wherein the presence of the residue correlates with an hypersusceptibility to treatment with the anti-viral treatment. In a preferred embodiment, the anti-viral treatment is a protease inhibitor.

5.11 Correlating Hypersusceptibility to One Anti-Viral Treatment with Hypersusceptibility to Another Anti-Viral Treatment

In another aspect, the present invention provides a method for using an algorithm of the invention to predict the effectiveness of an anti-viral treatment against a virus based on the genotypic susceptibility of the virus to a different anti-viral treatment. In one embodiment, the method comprises detecting, in a virus or a derivative of a virus, the presence or absence of one or more mutations correlated with hypersusceptibility to an anti-viral treatment and applying the rules of an algorithm of the invention to the detected mutations, wherein a score equal to, or greater than the genotypic cutoff score indicates that the virus is genotypically hypersusceptible to a different anti-viral treatment, and a score less than the genotypic cutoff score indicates that the virus is not genotypically hypersusceptible to a different anti-viral treatment. In another embodiment, the two anti-viral treatments affect the same viral protein. In another embodiment, the two anti-viral treatments are both protease inhibitors. Examples of protease inhibitors include, but are not limited to, saquinavir, ritonavir, indinavir, nelfinavir, amprenavir and lopinavir. In another embodiment, a mutation correlated with resistance to one protease inhibitor is also correlated with resistance to another protease inhibitor.

6. EXAMPLES

The following examples are provided to illustrate certain aspects of the present invention and not intended as limiting the subject matter thereof.

6.1 Example 1: Analysis of Patient Samples to Identify Hypersusceptibility-Associated Mutations

This example demonstrates a method of analyzing patient samples so as to identify mutations that are associated with hypersusceptibility to protease inhibitors.

In order to determine the relationship between an HIV-1 strain's protease sequence and its susceptibility to treatment with a protease inhibitor, a data set of patient plasma samples was analyzed genotypically as well as phenotypically. The phenotypic assay was conducted using the PHENOSENSE™ (Virologic, South San Francisco, CA) HIV assay (Petropoulos *et al.*, 2000, *Antimicrob. Agents Chemother.* 44:920-928; U.S. Patent Nos. 5,837,464 and 6,242,187). Plasma samples were collected from HIV-1-infected patients. Repeat samples from the same patient were removed to prevent possible bias resulting from unique combinations of mutations. In addition, samples with any resistance-selected mutation (see Table 2) in HIV-I protease or HIV-1 reverse transcriptase were excluded. This resulted in a data set of 1515 samples. Positions in the protease that varied in at least 1% of the sample set (*i.e.*, at least 15 samples) were considered in the analysis. IC_{50} values for several protease inhibitors were obtained for the HIV-1 from the patient samples. This was compared to the IC_{50} for the protease inhibitors against the NL4-3 (GenBank Accession No. AF324493) reference viral strain. Phenotypic data were expressed as "fold change" (or log fold change) in 50% inhibitory concentration (IC_{50}) of the protease inhibitor. The fold IC_{50} values were calculated by dividing the IC_{50} of the protease inhibitor against the HIV-1 from the patient plasma sample by the IC_{50} for the protease inhibitor against the NL4-3 (GenBank Accession No. AF324493) reference viral strain.

As seen in Figure 2, the fold change values observed were normally distributed for all the protease inhibitors. Table 3 shows the mean, median, 90^{th} and 10^{th} percentile values for the fold change (FC) for amprenavir ("APV"), indinavir ("IDV"), nelfinavir ("NFV"), ritonavir ("RTV"), saquinavir ("SQV") and lopinavir ("LPV").

Hypersusceptibility was defined as a fold change equal to or less than the 10^{th} percentile for each protease inhibitors' fold change distribution. Figure 3 shows inhibition curves for different protease inhibitors for the wild type or reference virus as well as for a sample with hypersusceptibility to the different protease inhibitors. Percent inhibition is plotted on the Y-axis and protease inhibitor concentration (in mM) is plotted on the X-axis. As can be seen in the figure, the curve for the sample with hypersusceptibility to the protease inhibitors (solid curve) is shifted to the left as compared to the curve for the wild type virus, indicating a lower IC_{50} (and thus an increased susceptibility) for the sample as compared to the wild-type.

Mean log-transformed fold-changes of samples with or without mutations at each position were compared by the t-test for comparison of means and the non-parametric

Kruskal-Wallis test. The numbers of samples defined as hypersusceptible with or without mutations at each position were compared using Fisher's Exact test. P-values of 0.05 or less were considered significant. Table 1 lists the positions that were found to be associated with hypersusceptibility for the different protease inhibitors by all three statistical tests. The mutations in the column "Positive Association" were over-represented in the samples found to be hypersusceptible to the protease inhibitor and those mutations in the "Negative Association" column were under-represented in the samples found to be hypersusceptible to the protease inhibitor. A virus with mutations at positions listed in the "Negative Association" column is less likely to have hypersusceptibility to protease inhibitors. The underlined positions were associated with the largest changes in mean fold change. Figure 4 shows the log FC for the wild type virus ("wt"), a mixture of samples containing the wild type virus and the indicated mutant ("mix") and a sample containing the indicated mutant ("mt") for the different protease inhibitors. Those mutants were selected that had the largest changes in mean fold change (*e.g.*, P39 for APV, E65 for IDV and so on).

Some of the mutations listed in Table 1 and associated with hypersusceptibility often occurred together, such as mutations at positions 69+89, 20+36, and 36+89. Since M36I, R41K, H69K, and L89M are signature mutations for non-B clade HIV, it is possible that non-B clade HIV may have increased susceptibility to some protease inhibitors. Figure 5 shows the protease inhibitor susceptibility for B clade and non-B clade viruses. As can be seen in the figure, the non-B clade viruses typically (with the exception of SQV) have higher susceptibility to protease inhibitors than do B clade viruses. This has important implications in the treatment of an individual infected with HIV-1. There is an increased likelihood that an individual infected with a non-B clade HIV will be hypersusceptible to a protease inhibitor as compared to an individual infected with a B clade HIV.

Figure 6 shows the protease inhibitor susceptibility for HIV split by clade. The clade HIV and the number of samples containing each clade are indicated to the right of the figure. As can be seen in the figure, different clade HIV have different susceptibilities to the different protease inhibitors. If the clade HIV infecting an individual is known, then the protease inhibitor to which that clade HIV is most susceptibility can be used.

6.2 Example 2: Effect of Mutations Associated with Hypersusceptibility to One Protease Inhibitor on Hypersusceptibility to Another Protease Inhibitor

In order to confirm that the PhenoSense™ assay performance was capable of discriminating small differences in phenotypic susceptibility within the range of variability observed in the wild-type viruses, the relationship between pairs of protease inhibitors was examined. If all of the variability was due to assay performance, one would expect to find no relationship between the FC for one drug with that of another. In contrast, a close relationship was observed for many protease inhibitor pairs. Table 4 summarizes the regression coefficients for each pair. Figure 7 shows the protease inhibitor susceptibility covariance for two pairs of protease inhibitors. As can be seen in the figure, the correlation between the protease inhibitors is very high (correlation coefficient, $R^2 = 0.69$ for IDV and RTV and $R^2 = 0.74$ for LPV and APV).

In order to determine whether hypersusceptibility to protease inhibitors was associated with reduced replication capacity ("RC") scatter plots (Figure 8) for each protease inhibitor vs. RC was generated using a data set of 402 viruses obtained from drug-naïve, recently infected patients lacking reduced susceptibility (FC > 2.5) to any drug or from a random sampling of a database sample with RC data of viruses also lacking reduced susceptibility (FC > 2.5) to any drug. As can be seen in the figure, while there is a weak association for some drugs (*e.g.*, SQV and LPV), in all cases there are many samples with low RC but normal (not HS) FC, and with high RC but HS. Thus the HS phenotype cannot always be explained by low RC.

All references cited herein are incorporated by reference in their entireties.

The examples provided herein, both actual and prophetic, are merely embodiments of the present invention and are not intended to limit the invention in any way.

Improving lopinavir genotype algorithm through phenotype correlations: novel mutation patterns and amprenavir cross-resistance

Neil T. Parkin, Colombe Chappey and Christos J. Petropoulos

Background: Current genotypic algorithms suggest that the HIV-1 protease inhibitors (PI) lopinavir (LPV) and amprenavir (APV) have distinct resistance profiles. However, phenotypic data indicate that cross-resistance is more common than expected.

Methods: Protease genotype (GT) and phenotype (PT) from 1418 patient viruses with reduced PI susceptibility and/or resistance-associated mutations (training data) were analyzed. Samples were classified as LPV resistant by GT (GT-R) if six or more LPV mutations were present, and by PT (PT-R) if the 50% inhibitory concentration (IC_{50}) fold-change (FC) was over 10.

Results: There were 182 samples (13%) that were GT-S but PT-R for LPV. A comparison of the mutation prevalence in PT-R/GT-S samples with that in PT-S/GT-S samples identified mutations associated with LPV PT-R. Several previously defined LPV mutations were found to have a stronger than average effect (e.g., M46I/L, I54V/T, V82A/F), and new variants at known positions (e.g., I54A/M/S, V82S) were identified. Other mutations, including known APV resistance mutations, were found to contribute to reduced LPV susceptibility. A new LPV genotypic interpretation algorithm was constructed that improved overall genotypic/phenotypic concordance from 80% to 91%. The algorithm demonstrated a concordance rate of 90% when tested on 523 new samples. Cross-resistance between APV and LPV was greater in samples with primary APV resistance mutations than in those lacking them.

Conclusions: The current LPV mutation score does not fully account for many resistant viruses. Consequently, cross-resistance between LPV and APV is underappreciated. Phenotypic results from large and diverse patient virus populations should be used to guide the development of more accurate GT interpretation algorithms.

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Keywords: HIV, protease, genotype, phenotype, resistance, lopinavir, amprenavir

Introduction

Protease inhibitors (PI) are important components of many highly active antiretroviral therapy (HAART) combination regimens [1]. Resistance to PI is one of the major challenges inherent in the selection of HAART regimens for treatment-experienced patients [2]. Lopinavir (LPV, ABT-378), co-formulated with ritonavir (LPV/r; Kaletra) has shown great promise in this area [3,4]. LPV plasma levels are increased greatly by ritonavir, resulting in antiviral activity against viruses with reduced PI susceptibility [5].

Genotypic (GT) and phenotypic (PT) correlates of resistance to LPV/r have been investigated *in vitro* and *in vivo*. *In vitro* passage of HIV-1 in the presence of LPV [6] resulted in the selection of viruses with reduced drug susceptibility and several mutations in the HIV protease. Analysis of viruses from PI-experienced patients (n = 112) entering phase III clinical trials of

See also p. 1077

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LPV/r in combination with other agents identified 23 mutations at 11 positions in protease associated with decreased LPV susceptibility (referred to here as LPV mutations): L10F, I, R, or V; K20M or R; L24I; M46I or L; F53L; I54L, T, or V; L63P; A71I, L, T, or V; V82A, F, or T; I84V; and L90M [7]. Subsequent analyses linked a reduction in LPV susceptibility, as measured by the fold change (FC) in 50% inhibitory concentration (IC_{50}) *in vitro* relative to a wild-type reference, of > 10-fold, or the presence of six or more LPV mutations, with diminished viral RNA responses during treatment with LPV/r [8]. These clinical data were used to establish the LPV susceptibility cut-off in PT tests (FC > 10) and of the LPV mutation score (six or more mutations) for predicting resistance on GT tests.

Since it is likely that there are multiple mutational pathways that could result in reductions in susceptibility > 10-fold, we wished to determine how well this genotypic interpretation algorithm predicts reduced susceptibility to LPV in a larger and more diverse sample set than previously examined. The present study compares genotypes and phenotypes from a database of patient samples that were submitted for routine drug resistance testing. The analysis identified numerous mutations associated with reduced LPV susceptibility that are not included in the initial genotypic algorithm. Importantly, mutations that are known to contribute to amprenavir (APV) resistance [9] were among these newly identified mutations.

Methods

PT was determined using PhenoSense HIV [10] and GT with GeneSeq HIV (ViroLogic, South San Francisco, California, USA). Both assays are performed by amplifying the pool of virus variants in the patient sample and use the same polymerase chain reaction product to construct resistance test vector DNA pools. Deduced amino acid sequences and drug susceptibility of patient viruses were compared with a reference virus strain (NL4-3) to identify mutations associated with alterations in drug susceptibility. Resistance test vector pools may contain mixtures of variants possessing both wild-type and mutant amino acids. The GeneSeq HIV assay detects minor virus populations at concentrations of ≥ 10–20%, depending on the mutation (GeneSeq HIV validation data, ViroLogic, data on file).

The FC thresholds used for defining a virus as sensitive (PT-S) or as having reduced susceptibility (PT-R) for LPV and APV were 10-fold [8] and 2.5-fold [11], respectively. Viruses with a FC below the threshold were considered to be sensitive to the drug, and viruses above the threshold were considered to have reduced drug susceptibility (i.e., the drug has reduced probability of antiviral activity, although it may retain partial activity). A LPV genotype mutation score was calculated for each sample by counting the number of defined LPV resistance-associated mutations (L10F, I, R, or V; K20M or R; L24I; M46I or L; F53L; I54L, T, or V; L63P; A71I, L, T, or V; V82A, F, or T; I84V; L90M) identified in the sample. Viruses were classified as resistant to LPV by GT (GT-R) if the LPV mutation score was six or more [8]. For APV, samples were classified as GT-R if any of the primary APV resistance-associated mutations (V32I, I50V, I54L, I54M, or I84V) were present [9]. Mixtures of mutant and wild-type amino acids at any resistance-associated position were counted as mutant.

The primary analysis dataset (training data) included 2038 LPV phenotypes and protease genotypes, representing unique patient samples tested between January 2000 and September 2001. Wild-type viruses [FC < 2-fold by PT and absence of mutations by GT at any of the protease amino acid positions 30, 32, 46, 48, 50, 54, 82 (except V82I), 84, 88, or 90] were removed, leaving 1418 samples.

A second set of patient samples (validation data) was assembled containing results from 1022 patient samples tested between October 2001 and January 2002, and excludes samples from patients included in the training dataset. After removal of wild-type viruses, 523 samples remained.

The relative prevalence of various individual mutations in LPV PT-R or PT-S samples was compared by constructing 2 × 2 contingency tables; odds ratios (OR) were calculated to evaluate the association between individual mutations and LPV resistance, and statistical significance was tested using Fisher's exact test. Because of the multiple comparisons performed, mutations were considered significantly different between groups only if the P value was ≤ 0.01. At positions with significant variability in the amino acid present (for example, 54A, L, M, S, T, or V), each variant was tested separately but compared with samples with all other variants at that position (i.e., I54A was compared with all other samples including those with 54I, L, M, S, T, or V). To account for the possibility that some mutations have a stronger effect than others, weighting factors were tested for mutations that were most strongly associated with phenotypic resistance, taking into consideration patterns of linkage between mutations.

Results

To obtain an initial estimate of the accuracy of the

LPV mutation score [7,8] as a predictor of reduced LPV susceptibility, LPV FC was plotted against the mutation score for 1418 viruses derived from patient plasma samples (Fig. 1a). While there was clearly a trend for susceptibility to decrease as the mutation score increased (linear regression coefficient $r^2 = 0.50$), there was considerable variability in LPV susceptibility within each mutation score category. Although the phenotypic assay provided a more quantitative assessment of drug susceptibility than the genotypic assay, both test results were categorized as either sensitive (S; FC < 10 or fewer than six LPV mutations) or reduced susceptibility (R, or resistant; FC > 10 or six or more

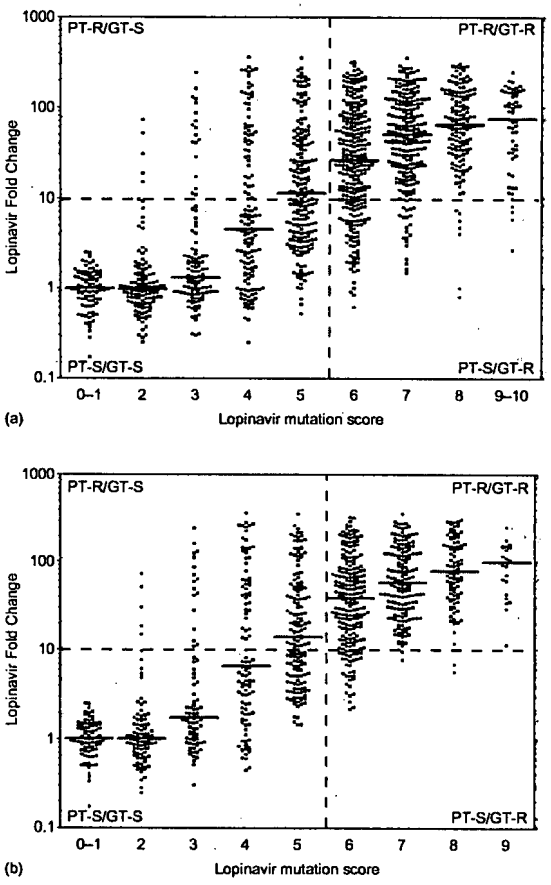

Fig. 1. Scatter plot of lopinavir susceptibility versus mutation score. Lopinavir fold change (in 50% inhibitory concentration *in vitro* relative to a wild-type reference) is plotted on a logarithmic scale; mutations in the original lopinavir mutation score (see Methods) were counted and plotted on the *x* axis. Dashed lines indicate thresholds for susceptibility/reduced susceptibility. Horizontal lines for each group of samples defined by mutation score represent median fold change for that group. (a) Mixtures of wild type and mutant amino acids were counted as mutant. (b) Samples containing mixtures of wild type and mutant amino acids at positions 46, 53, 82, 84 or 90 were removed. GT, genotype; PT, phenotype; S, sensitive; R, reduced susceptibility.

LPV mutations) to simplify the determination of concordant or discordant test results. Based on this categorical analysis, the overall concordance between PT and GT was 79%. Another 13% of the samples were PT-R/GT-S and 8% were PT-S/GT-R (Table 1).

Further examination revealed specific causes for discordant test results. The presence of mixtures of mutant and wild-type virus was a significant cause of PT-S/GT-R discordance. If the relative proportion of mutant variants present in the patient sample is low, the PT result from the mixed pool of viruses could underestimate the drug susceptibility of the resistant virus. Exclusion of all viruses with at least one mixture at position 46, 53, 82, 84 or 90 resulted in a significant reduction in the number of PT-S/GT-R samples (39 versus 110), and a modest increase in the overall correlation between PT and GT ($r^2 = 0.55$). The remaining PT-S/GT-R samples clustered near the PT and GT sensitivity thresholds (Fig. 1b).

Although removal of samples with virus mixtures reduced the percentage of PT-S/GT-R samples, the overall concordance improved only slightly (80%), with 16% of viruses being PT-R/GT-S. We hypothesized that these discordant viruses contained previously uncharacterized mutations that contribute to reduced LPV susceptibility. To identify such mutations, the frequency was compared for any protease mutation in the PT-R/GT-S and the PT-S/GT-S groups that was detected in > 1% of all samples. To assess the strength of the association between each mutation and LPV FC > 10, the mutations were ranked by calculating the OR for identifying the mutation in the PT-R compared with the PT-S group (Table 2). The OR for mutations with $P < 0.01$ ranged from < 1 (e.g., D30N and N88D; not shown) to high values that could not be calculated because no samples with the mutation had FC < 10; mutations were arbitrarily considered to be associated with LPV resistance when the OR was > 2. The resulting list of mutations fell into three categories: those included in the initial LPV mutation score, new amino acid variants at positions in the LPV mutation score, and novel mutations at positions not described in the LPV mutation score.

Mutations in the current LPV mutation score that are over-represented in PT-R/GT-S viruses included L10F or I; K20M; M46I or L; I54T or V; and V82A or F. This suggests that these mutations, particularly I54T/V and V82A/F (OR > 9), may have a stronger effect on LPV phenotype than others in the algorithm. New amino acid variants at known positions included K20I; I54A, M, and S; L63T; and V82S. These mutations are likely to have a similar impact on LPV susceptibility as other previously identified resistance mutations at the same amino acid positions, but since they are less prevalent, they were not identified in

Table 1. Protease genotype–phenotype discordance rates for lopinavir.

	No.	PT-R, GT-R	PT-S, GT-S	PT-R, GT-S	PT-S, GT-R
All samples	2038	637 (31%)	1109 (54%)	182 (9%)	110 (5%)
Excluding wild type[a]	1418	637 (45%)	489 (34%)	182 (13%)	110 (8%)
Excluding mixtures[b]	1014	458 (45%)	359 (35%)	158 (16%)	39 (4%)

GT, genotype; PT, phenotype; S, sensitive; R, reduced susceptibility.
[a]Samples with all protease inhibitors fold change (50% inhibitory concentration *in vitro* relative to a wild-type reference) < 2 and no primary protease inhibitors mutations were excluded.
[b]Samples with at least one mixture at positions 46, 53, 82, 84, or 90 were excluded.

Table 2. Mutations associated with lopinavir protease phenotype resistance in genotype susceptibility samples.

		No. with fold change[b]			
Category[a]	Mutation	< 10	> 10	OR[c]	*P* value
1	I54T	0	5	≫	0.0025
	I54V	9	52	13.1	< 0.0001
	V82A	19	78	9.3	< 0.0001
	V82F	2	8	9.1	0.0016
	K20M	5	10	4.5	0.0037
	L10F	18	25	3.2	0.0001
	M46L	19	20	2.4	0.0060
	M46I	57	58	2.3	< 0.0001
	L10I	118	105	2.0	< 0.0001
2	I54A	0	24	≫	< 0.0001
	I54S	0	16	≫	< 0.0001
	V82S	0	8	≫	0.0001
	I54M	8	21	6.0	< 0.0001
	L63T	5	12	5.5	0.0006
	K20I	16	25	3.6	0.0000
3	G48M	0	4	≫	0.0085
	L89I	0	4	≫	0.0085
	I50V	1	21	47.7	< 0.0001
	K43T	2	19	21.6	< 0.0001
	G48V	9	53	13.4	< 0.0001
	E34Q	2	10	11.4	0.0002
	I47V	4	19	10.8	< 0.0001
	G16E	7	22	7.1	< 0.0001
	L33F	16	38	5.4	< 0.0001
	V32I	8	18	5.1	< 0.0001
	T74S	17	28	3.7	< 0.0001
	L89M	7	11	3.6	0.0074
	G73T	10	13	3.0	0.0095
	Q58E	15	17	2.6	0.0087

[a]Category 1, mutations in existing lopinavir mutation score; category 2, new amino acid substitutions at positions in existing lopinavir mutation score; category 3, new mutations, i.e. not in existing lopinavir mutation score.
[b]Fold change in 50% inhibitory concentration *in vitro* relative to a wild-type reference.
[c]Odds ratio (OR) calculated by dividing the percentage of phenotype reduced susceptibility samples with the mutation by the percentage of phenotype sensitive samples with the mutation.

earlier studies performed with smaller numbers of patient samples.

The list of LPV resistance-associated mutations at novel amino acid positions that are not part of the current LPV mutation score includes several recognized PI resistance mutations such as G16E, V32I, L33F, I47V, G48M/V, I50V and G73T [12]. G16E, V32I, and I47V have been selected by *in vitro* passage of HIV in cell cultures in the presence of LPV [6]. Other mutations in this category (E34Q, K43T, Q58E, T74S, and L89I/M) have not previously been associated with PI resistance.

Based on these findings, a modified LPV mutation score algorithm was developed that included the mutations in Table 2 (an additional 20 mutations and 12 positions) and increased the weight assigned to positions 50, 54, and 82. The optimum number of mutations required for GT-R was seven, determined by evaluating the overall concordance rate in the training dataset (without mixtures). The resulting algorithm demonstrated 91% concordance with the training data, compared with 80% using the original LPV mutation score. The new algorithm was also tested on a validation dataset of 523 previously unanalyzed samples after removing samples with mixtures of mutant and wild-type viruses at amino acid positions in the original LPV mutation score. In this dataset, the concordance increased from 84% with the original algorithm to 90% with the new algorithm.

To assess the accuracy of the new algorithm further, the training and validation datasets were combined and a scatter plot of LPV FC versus new mutation score was generated (Fig. 2). Compared with the original LPV mutation score, less variation in LPV susceptibility was observed within any given mutation score, resulting in a higher correlation coefficient with the new algorithm ($r^2 = 0.80$ compared with 0.62). The two prominent outlier viruses (indicated by arrows in Fig. 2) with high-level resistance to LPV and four mutations were found to contain the rare I47A mutation. Both samples also contained V32I and M46I, as well as several polymorphisms. I47A was selected *in vitro* in the presence of high LPV concentrations [6].

Several of the LPV resistance mutations identified in this study (V32I, M46I/L, I47V, I50V, I54M) are also associated with resistance to APV [9]. The effect of these mutations alone and in combination with each other and with I84V on LPV and APV susceptibility is summarized in Table 3. The median APV FC was 9.5-

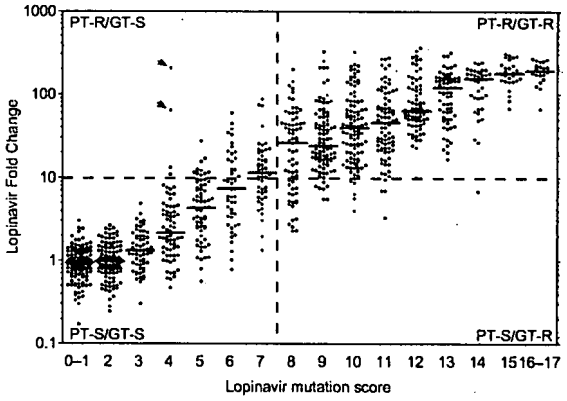

Fig. 2. Scatter plot of lopinavir susceptibility versus new mutation score. Lopinavir fold change (in 50% inhibitory concentration *in vitro* relative to a wild-type reference) is plotted on a logarithmic scale; mutations in the updated lopinavir mutation score (see text) were counted, additional weighting given to mutations at positions 50, 54 and 82, and plotted on the *x* axis. Dashed lines indicate thresholds for susceptibility/reduced susceptibility. Horizontal lines for each group of samples defined by mutation score represent median fold change for that group. Arrows indicate two outlier samples, both of which contain the I47A mutation. GT, genotype; PT, phenotype; S, sensitive; R, reduced susceptibility.

Table 3. Effect of amprenavir mutations on lopinavir and amprenavir susceptibility.

		Median fold change[b]	
Genotype group[a]	No.	Lopinavir	Amprenavir
Wild type (all primary positions)	623	0.6	0.6
32	26	12	9.5
47	8	191	26
50	42	54	20
54[c]	9	160	22
84	247	7.1	11
32, 47	23	7.5	12
32, 84	3	124	51
46[d], 54	16	138	84
47, 54	3	88	42
47, 84	4	28	12
54, 84	22	93	67
32, 47, 54	17	146	40
46, 54, 84	29	30	47
47, 54, 84	3	20	24
32, 46, 47, 54	4	208	130
32, 47, 54, 84	5	227	130
32, 46, 47, 54, 84	6	200	130

[a]Each group of samples contains the indicated mutations and lacks any of the others listed in the table; therefore, except for the wild-type group, they may also contain other mutations not listed (e.g., V82A, L90M).
[b]Fold change in 50% inhibitory concentration *in vitro* relative to a wild-type reference.
[c]I54L or M only.
[d]M46I or L.

fold or higher in each group. The median LPV FC generally paralleled, and often exceeded, the APV FC in these groups. Figure 3 demonstrates cross-resistance between the drugs in a bivariate scattergram of LPV versus APV FC for the combined dataset. The correlation of phenotypic susceptibilities between these two PI drugs was greater among viruses meeting the criteria for APV resistance by GT (black squares; $r^2 = 0.52$) than among those that were GT-S (grey dots; $r^2 = 0.39$; all samples with FC < 2.5 for either PI were excluded from the correlations to avoid skewing the regression to samples susceptible to both drugs). Of all the viruses that were PT-R to either PI, 76% were resistant to both PI. Among APV GT-R viruses, 95% were PT-R for APV and 80% were PT-R for LPV.

Discussion

The development of GT interpretation algorithms for drugs that require multiple mutations for the acquisition of clinically relevant levels of resistance is complex. Such algorithms are highly dependent on the number of viruses included in the analysis and the previous treatment experience of the patients from which the viruses are derived. Additionally, since cross-resistance within a drug class is a common phenomenon [13–16],

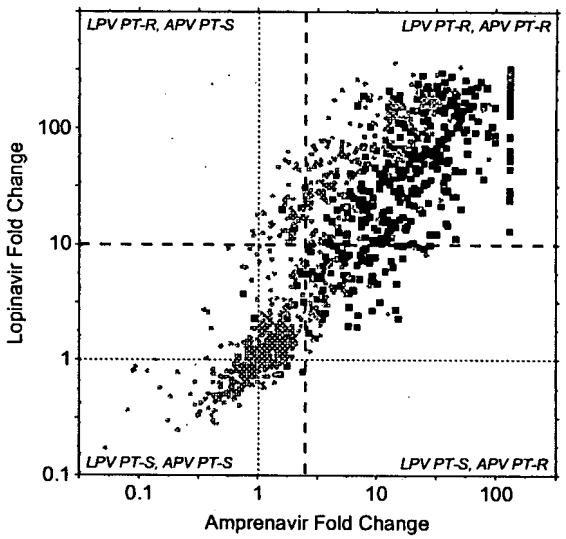

Fig. 3. Scatter plot of lopinavir versus amprenavir susceptibility. Fold change (in 50% inhibitory concentration *in vitro* relative to a wild-type reference) is plotted on a logarithmic scale for both drugs. Samples with a primary amprenavir resistance mutation (V32I, I50V, I54L or M, or I84V) are indicated by black squares ($r^2 = 0.52$); samples lacking such mutations are indicated by gray dots ($r^2 = 0.39$). APV, amprenavir; LPV, lopinavir; GT, genotype; PT, phenotype; S, sensitive; R, reduced susceptibility.

mutation patterns that confer resistance to one drug may not be completely defined solely by evaluation of viruses from patients treated with that drug. This often leads to genotype interpretation rules that are limited in their ability to predict phenotypic resistance. If a clinically relevant threshold for defining phenotypic drug susceptibility is set and a large set of PT and GT data from samples with diverse patterns of mutations is available, as in the present study, significant improvements can be made to a GT interpretation algorithm that had been initially derived from a limited number of viruses and patients with limited types of treatment experience.

The analysis revealed three categories of mutations that were either underemphasized or not included in the original LPV GT interpretation algorithm. It was not surprising to find that some mutations in the current algorithm contribute to LPV resistance more strongly than others (Table 2), since many of the positions in the LPV mutation score are not in close proximity to the active site where the drug binds (e.g., 63, 71). However, not all mutations near the active site are associated with PT-R/GT-S discordance (e.g., L24I or I84V). Other factors, such as how the drug interacts with various amino acid residues, are likely to influence the relative importance in determining resistance. New variants at known mutation positions, and other previously uncharacterized resistance mutations, are likely to have been missed during the development of the initial GT algorithm because their prevalence is low and a relatively small population was studied, or because they are selected by drugs such as APV, which was not widely utilized in treatment regimens at the time that the original LPV clinical studies were conducted [7].

The importance of the APV-selected mutation I50V in determining susceptibility to LPV has been recently reported [17,18]. However, viruses from patients failing APV as their first PI, which develop I50V, do not always have LPV FC > 10-fold [9]. This apparent discrepancy can be explained by the fact that the I50V-containing samples analysed in the present study also contained other primary PI mutations.

There are several limitations to this study. A treatment history was available for relatively few of the patients studied, making it difficult to examine the patterns of resistance in all patients who have been exposed to a particular PI. Since a second cut-off above which a sample can be classified as being truly resistant has yet to be firmly identified, the samples cannot be divided into more than two groups, such as 'susceptible', 'resistant' and 'partially resistant' by PT or GT. However, this approach has the potential of providing more information by distinguishing 'partial response' from 'no response' based on GT or PT and is currently being investigated. In addition, the cut-off used to define phenotypic resistance to APV (2.5-fold) was not derived from clinical outcome studies. However, this threshold is probably relevant for several reasons. Strong correlations exist between PT results using this cut-off and clinical response data in cohorts that included patients receiving APV [19,20]. Reductions in susceptibility to APV observed in patients who experienced viral load rebound while using APV as their first PI are modest (as low as 2–3-fold) [9]. Finally, the 99th percentile for the distribution of APV FC in genotypically wild-type viruses using the PhenoSense assay is 2.1 [21]. The clinically relevant PT cut-off, as well as the number of PI mutations required for resistance, is expected to be higher for patients treated with ritonavir-boosted APV therapy.

Isaacson and colleagues recently presented an independent analysis on the impact of various protease mutations on viral load response to LPV in 792 heavily PI-experienced patients [22]. This study provides complementary information and independent confirmation of the importance of mutations at positions 10, 20, 33, 47, 48, 50, 54, and 82 for LPV resistance. In addition, another recent study found that mutations emerging following viral load rebound in 21 patients undergoing salvage treatment with LPV included variants at positions 33, 47, 48, 50, 58, and 73 [23]. A third study also reported the emergence of mutations of positions 10, 20, 33, 46, 47, 50, 54, and 82 following salvage therapy with LPV [24]. Although other positions identified in these three studies were not identified here (e.g., 36, 77), the significant overlap between studies strongly suggests that our results are clinically significant and that these additional mutations should be included in LPV genotypic interpretation algorithms. The analysis of large numbers of phenotypes and genotypes in the context of a clinically significant PT cut-off value is an important means of improving the accuracy of drug susceptibility predictions generated using genotypic algorithms that are based on limited data from clinical trials in relatively homogeneous patient groups.

Acknowledgements

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Table 5. Protease and reverse transcriptase genotypes of virus from treatment failures.

Subject no.	Time	Resistance-associated mutations	
		Protease	Reverse transcriptase
1	baseline	L10I, K20I, M36I, I54V, L63P, A71V, V82T	D67N, T69D, K70R, M184V, T215F, K219Q
	4 weeks	L10I, K20I, M36I, I54V, L63P, A71V, V82T	D67N, T69D, K70R, M184V, T215F, K219Q
	5 weeks	L10I, K20I, M36I, I54V, L63P, A71V, V82T	D67N, T69D, K70R, Y181Y/C, M184M/V, T215F, K219Q, F227F/L
	11 weeks	L10I, K20I, M36I, I54V, L63P, A71V, V82T	D67N, T69D, K70R, V106A, T215F, K219Q, F227L
2	baseline	L63P, G73G/C, L90L/M	M41L, T69A/D/V, V118V/I, M184V, L210W, T215Y
	10 weeks	L10L/I, L63P, G73C, L90M	M41L, A98G, M184V, L210W, T215Y
	14 weeks	L10L/I, M46M/L, L63P, G73C, L90M	M41L, A98G, M184V, L210W, T215Y
	18 weeks	L10L/I, M46M/L, L63P, G73C, L90M	M41L, A98G, M184V, L210W, T215Y
	22 weeks	L10L/I, M46M/L, L63P, G73C, V77V/I, L90M	M41L, A98G, M184M/V, L210W, T215Y
6	baseline	L10I, M46I, L63P, A71V, L90M	D67G, K70R, M184V, K219Q
	5 weeks	L10I, M46I, L63P, A71V, L90M	D67G, K70R, M184V, T215T/A, K219Q
	9 weeks	L10I/V, K20K/R, M36M/I, M46M/L, L63P, A71V V82V/A, I84I/V, L90M	D67G, K70R, M184V, T215T/A/I/V, K219Q
	18 weeks	L10I/V, K20R, M36M/I, M46M/L, G48G/V, L63P, A71V, V82V/A, L90M	D67G, K70R, M184V, T215T/A/I/V, K219Q
7	baseline	L10L/I, L24I, M46I/L, I54A/V, L63P, A71L/V, V82A, L90L/M	L74V, M184V
	8 weeks	L10I, G48G/V, I54V, L63P, A71V, V82A, L90M	L74V, M184V
	16weeks	L10I, G48V, I54V, A71V, V77V/I, V82A, L90M	L74V, M184V
	36 weeks	L10I, G48V, I54V, A71V, V77I, V82A, L90M	L74V, M184V
10	baseline	K20I, L63P, V77I	M184V
	13 weeks	K20I, L63P, A71A/T, G73G/S, V77I, L90M	M184V
	17 weeks	K20K/I, L63P, A71A/I/T/V, G73G/S, V77I, L90M	M184V, T215T/N/S/Y
	26 weeks	K20I, L63P, A71A/I/T/V, G73G/S, V77I , L90M	M184V, T215Y
	33 weeks	K20I, L63P, A71A/I/T/V, G73G/S, V77I, L90M	M184V, T215Y
12	baseline	L10I, L24I, M46I, I54V, L63P, V82A	D67N, T69D, K70R, M184V, T215F, K219Q
	36 weeks	L10I, L24I, M46I, I54V, L63P, V82A	D67N, T69D, Y181C, M184M/V, T215F, K219Q
	48 weeks	L10I, L24I, M46I, I54V, L63P, V82A	D67N, T69D, K70K/R, Y181C, M184M/V, T215F, K219Q
14	baseline	L10I, M46I, L63L/P, G73A/T, V77I, L90M	M41L, L74I, M184V, L210W, T215Y
	4 weeks	L10I, M46I, L63L/P, G73S/T, V77I, L90M	M41L, A62A/V, L74I, Y181Y/C, M184V, L210W, T215Y, M230M/L
	8 weeks	L10I, M46I, L63P, G73S, V77I, L90M	M41L, L74I, Y181C, M184V, L210W, T215Y, M230L
	16 weeks	L10I, M46I, L63P, G73S/T, V77I, L90M	M41L, L74I, Y181C, M184V, L210W, T215Y, M230L
19	baseline	L10I, L24I, M46L, I54V, L63P, A71V, V82A	M41L, M184V, T215Y
	2 weeks	L10I, L24I, M46L, I54V, L63P, A71V, V82A	M41L, M184V, T215Y
	4 weeks	L10I, L24I, M46L, I54V, L63P, A71V, V82A	M41L, L74V, K103N, Y181C, M184V, T215Y
	8 weeks	L10I, L24I, M36M/I, M46L, I54V, L63P, A71V, V82A	M41L, L74V, K103N, Y181C, M184V, T215Y
	12 weeks	L10I, L24I, M36M/I, M46L, I54V, L63P, A71V, V82A	M41L, L74V, K103N, Y181C, M184V, T215Y
20	baseline	M46L, I54I/V, L63P, V82V/A, L90L/M	M41L, M184V, T215Y
	12 weeks	M46L, I54V, L63P, V82A, L90L/M	M41L, M184V, T215Y
	16 weeks	M46L, I54I/V, L63P, V82V/A, L90L/M	M41L, M184V, T215Y
	24 weeks	M46L, L63P, L90M	M41L, D67N, K103N, M184M/V, T215Y
	28 weeks	M46L, L63P, L90M	M41L, D67N, K103N, M184V, T215Y
	36 weeks	M46L, L63P, L90M	M41L, D67N, K103N, M184M/V, T215Y
21	baseline	L10I, M46M/I, I54I/V, L63Q, V82A	K103N, P225H
	12 weeks	L10I, G48V, L63Q, V82A	D67D/G, K103N, M184M/I, P225H
	16 weeks	L10L/I, L24L/I/M, M46M/I, G48G/V, L63Q, V82A	K103N, M184M/I, P225H
	20 weeks	L10I, G48V, L63Q, V82A	D67D/G, K103N, M184I, P225H
	24 weeks	L10I, G48V, L63Q, V82A	D67D/G, K103N, M184I/V, P225H

[a]Major resistance-associated mutations [23] are listed, as changes compared to the NL4-3 reference sequence. Mixtures are indicated by listing all possible amino acids at the codon in question separated by a slash.
Mutations in bold type represent changes from that patient's baseline genotype

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=> e parkin neil t/in
E1            8     PARKIN MICHAEL W/IN
E2            2     PARKIN NEIL JAMES/IN
E3           27 --> PARKIN NEIL T/IN
E4            5     PARKIN NIGEL/IN
E5            1     PARKIN PAT/IN
E6            2     PARKIN PAT D/IN
E7            2     PARKIN PAUL C/IN
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E9            2      PARKIN ROB/IN
E10           1      PARKIN ROBERT/IN
E11           2      PARKIN ROBERT E/IN
E12           1      PARKIN ROBERT EDWARD/IN

=> s e3
L1           27 "PARKIN NEIL T"/IN

=> s l1 and (PR/clm or protease/clm)
          8975 PR/CLM
          8461 PROTEASE/CLM
L2          12 L1 AND (PR/CLM OR PROTEASE/CLM)

=> s l2 and (HIV?/clm or human immunodeficiency virus/clm)
          7950 HIV?/CLM
        109439 HUMAN/CLM
          3127 IMMUNODEFICIENCY/CLM
         19686 VIRUS/CLM
          2213 HUMAN IMMUNODEFICIENCY VIRUS/CLM
                 ((HUMAN(W)IMMUNODEFICIENCY(W)VIRUS)/CLM)
L3          11 L2 AND (HIV?/CLM OR HUMAN IMMUNODEFICIENCY VIRUS/CLM)

=> s l3 and (hypersusceptibil?/clm or increase? susceptibilit?/clm)
             2 HYPERSUSCEPTIBIL?/CLM
        250620 INCREASE?/CLM
          3919 SUSCEPTIBILIT?/CLM
           139 INCREASE? SUSCEPTIBILIT?/CLM
                 ((INCREASE?(W)SUSCEPTIBILIT?)/CLM)
L4            3 L3 AND (HYPERSUSCEPTIBIL?/CLM OR INCREASE? SUSCEPTIBILIT?/CLM)

=> d l4,cbib,clm,1-3

L4   ANSWER 1 OF 3  USPATFULL on STN
2004:138933 Compositions and methods for determining the susceptibility of a
    pathogenic virus to protease inhibitors.
    Parkin, Neil T., Belmont, CA, UNITED STATES
    Paxinos, Ellen, San Jose, CA, UNITED STATES
    Chappey, Colombe, San Francisco, CA, UNITED STATES
    Wrin, Mary T., Fremont, CA, UNITED STATES
    Gamarnik, Andrea, Chandler, AZ, UNITED STATES
    Petropoulos, Christos J., Half Moon Bay, CA, UNITED STATES
    US 2004106106 A1 20040603
    APPLICATION: US 2003-612600  A1  20030701 (10)
    PRIORITY: US 2002-393234P  20020701 (60)
    DOCUMENT TYPE: Utility; APPLICATION.
CAS INDEXING IS AVAILABLE FOR THIS PATENT.
CLM    What is claimed is:
       1. A method for determining whether a HIV-1 has an increased
       likelihood of being hypersusceptible to treatment with a protease
       inhibitor, comprising: detecting whether the protease encoded by said
       HIV exhibits the presence or absence of a mutation associated with
       hypersusceptibility to treatment with said protease inhibitor at
       amino acid position 16, 20, 33, 36, 37, 39, 45, 65, 69, 77, 89 or 93 of
       an amino acid sequence of said protease, wherein the presence of said
       mutation indicates that the HIV has an increased likelihood of being
       hypersusceptible to treatment with the protease inhibitor, with the
       proviso that said mutation is not L33F.

       2. The method of claim 1, wherein the protease has a sequence that is
       greater than 80% identical to SEQ ID NO: 1.

       3. A method for determining whether an individual infected with HIV-1
       has an increased likelihood of being hypersusceptible to treatment with
       a protease inhibitor, comprising detecting, in a sample from said
       individual, the presence or absence of a mutation associated with
       hypersusceptibility to treatment with said protease inhibitor at
       amino acid position 16, 20, 33, 36, 37, 39, 45, 65, 69, 77, 89 or 93 of
       the amino acid sequence of the protease of the HIV-1, wherein the
       presence of said mutation indicates that the individual has an increased
       likelihood of being hypersusceptible to treatment with the protease
       inhibitor, with the proviso that said mutation is not L33F.

       4. The method of claim 3, wherein the protease has a sequence that is
       greater than 80% identical to SEQ ID NO: 1.

       5. An isolated oligonucleotide between about 10 and about 40 nucleotides
       long encoding a portion of an HIV protease that comprises a mutation
       at amino acid position 16, 20, 33, 36, 37, 39, 45, 65, 69, 77, 89 or 93
       of an amino acid sequence of said protease in said human
       immunodeficiency virus, wherein the mutation is associated with
       hypersusceptibility to a protease inhibitor, with the proviso that
       said mutation is not L33F.
```

6. The method of claim 1, wherein said **protease** inhibitor is amprenavir.

7. The method of claim 6, wherein said amino acid position is 20, 36, 39, 65, 69, 77 or 89.

8. The method of claim 1, wherein said **protease** inhibitor is indinavir.

9. The method of claim 8, wherein said amino acid position is 16, 39 or 65.

10. The method of claim 1, wherein said **protease** inhibitor is nelfinavir.

11. The method of claim 10, wherein said amino acid position is 16, 39, 65, 69 or 89.

12. The method of claim 1, wherein said **protease** inhibitor is ritonavir.

13. The method of claim 12, wherein said amino acid position is 39, 65 or 93.

14. The method of claim 1, wherein said **protease** inhibitor is saquinavir.

15. The method of claim 14, wherein said amino acid position is 33, 37, 45, 65 or 77, with the proviso that the mutation at amino acid position 33 is not 33F.

16. The method of claim 1, wherein said **protease** inhibitor is lopinavir.

17. The method of claim 16, wherein said amino acid position is 33, 39, 65, 77 or 93, with the proviso that the mutation at amino acid position 33 is not 33F.

18. The method of claim 3, wherein the individual is undergoing or has undergone prior treatment with an anti-viral drug.

19. The method of claim 1, wherein the method comprises detecting the presence or absence of a mutation associated with **hypersusceptibility** to treatment with said **protease** inhibitor at at least 2, 3, 4, 5, 6, 7, 8, 9, 10, 11 or 12 of the amino acid positions.

20. A method for determining whether a **HIV**-1 has a decreased likelihood of being hypersusceptible to a **protease** inhibitor, comprising: detecting whether the **protease** encoded by said **HIV**-1 exhibits the presence or absence of a mutation negatively associated with **hypersusceptibility** to said **protease** inhibitor at amino acid position 10, 15, 36, 41, 57, 60, 63, 71 or 93 of an amino acid sequence of said **protease**, wherein the presence of said mutation indicates that the **HIV** has a decreased likelihood of being hypersusceptible to the **protease** inhibitor.

21. A method for determining whether an individual infected with **HIV**-1 has a decreased likelihood of being hypersusceptible to treatment with a **protease** inhibitor, comprising detecting, in a sample from said individual, the presence or absence of a mutation negatively associated with **hypersusceptibility** to treatment with said **protease** inhibitor at amino acid position 10, 15, 36, 41, 57, 60, 63, 71 or 93 of the amino acid sequence of the **protease** of the **HIV**-1, wherein the presence of said mutation indicates that the individual has a decreased likelihood of being hypersusceptible to treatment with the **protease** inhibitor.

L4 ANSWER 2 OF 3 USPATFULL on STN
2003:159244 Means and methods for monitoring protease inhibitor antiretroviral therapy and guiding therapeutic decisions in the treatment of HIV/AIDS.
Parkin, Neil T., Belmont, CA, UNITED STATES
Ziermann, Rainer A., San Mateo, CA, UNITED STATES
US 2003108857 A1 20030612
APPLICATION: US 2001-874472 A1 20010604 (9)
DOCUMENT TYPE: Utility; APPLICATION.
CAS INDEXING IS AVAILABLE FOR THIS PATENT.
CLM What is claimed is:
1. A method of assessing the effectiveness of **protease** antiretroviral therapy of an **HIV**-infected patient comprising: (a) collecting a plasma sample from the **HIV**-infected patient; (b) evaluating whether the plasma sample contains nucleic acid encoding **HIV protease** having a mutation at codon 88; and (c) determining **increased susceptibility** to amprenavir.

2. The method of claim 1, wherein the mutation at codon 88 codes for a serine (S).

3. The method of claim 1, wherein the **HIV**-infected patient is being treated with an antiretroviral agent.

4. A method of assessing the effectiveness of **protease** antiretroviral therapy of an **HIV**-infected patient comprising: (a) collecting a plasma sample from the **HIV**-infected patient; (b) evaluating whether the plasma sample contains nucleic acid encoding **HIV protease** having a mutation at codon 88 and additional mutations at codons 63 and/or 77 or a combination thereof; and (c) determining decreased susceptibilty to nelfinavir and indinavir and **increased susceptibility** to amprenavir.

5. The method of claim 4, wherein the mutation at codon 63 codes for a proline (P) or a glutamine (Q) and the mutation at codon 77 codes for an isoleucine (I).

6. The method of claim 4, wherein the **HIV**-infected patient is being treated with an antiretroviral agent.

7. A method of assessing the effectiveness of **protease** antiretroviral therapy of an **HIV**-infected patient comprising: (a) collecting a plasma sample from the **HIV**-infected patient; (b) evaluating whether the plasma sample contains nucleic acid encoding **HIV protease** having a mutation at codon 88 and additional mutations at codons 63, 77 and/or 46 or a combination thereof; and (c) determining decreased susceptibilty to nelfinavir and indinavir and **increased susceptibility** to amprenavir.

8. The method of claim 7, wherein the mutation at codon 63 codes for a proline (P) or a glutamine (Q), the mutation at codon 77 codes for an isoleucine (I). and the mutation at codon 46 codes for a leucine (L) or an isoleucine (I).

9. The method of claim 7, wherein the **HIV**-infected patient is being treated with an antiretroviral agent.

10. A method of assessing the effectiveness of **protease** antiretroviral therapy of an **HIV**-infected patient comprising: (a) collecting a plasma sample from the **HIV**-infected patient; (b) evaluating whether the plasma sample contains nucleic acid encoding **HIV protease** having a mutation at codon 88 and additional mutations at codons 63, 77, 46, 10, 20, and/or 36 or a combination thereof; and (c) determining decreased susceptibilty to nelfinavir and indinavir and **increased susceptibility** to amprenavir.

11. The method of claim 10, wherein the mutation at codon 63 codes for a proline (P) or a glutamine (Q), the mutation at codon 77 codes for an isoleucine (I), the mutation at codon 46 codes for a leucine (L) or an isoleucine (I), the mutation at codon 10 codes for a isoleucine (I) or a phenylalanine (F), the mutation at 20 codes for a threonine (T) or a methionine (M) or an arginine (R), and the mutation at 36 codes for an isoleucine (I) or a valine (V).

12. The method of claim 10, wherein the **HIV**-infected patient is being treated with an antiretroviral agent.

13. A method for evaluating the biological effectiveness of a candidate **HIV** antiretroviral drug compound comprising: (a) introducing a resistance test vector comprising a patient-derived segment further comprising a mutation at codon 88 and an indicator gene into a host cell; (b) culturing the host cell from step (a); (c) measuring the indicator in a target host cell; and (d) comparing the measurement of the indicator from step (c) with the measurement of the indicator measured when steps (a)-(c) are carried out in the absence of the candidate antiretroviral drug compound; wherein a test concentration of the candidate antiretroviral drug compound is present at steps (a)-(c); at steps (b)-(c); or at step (c).

14. A method for evaluating the biological effectiveness of a candidate **HIV** antiretroviral drug compound comprising: (a) introducing a resistance test vector comprising a patient-derived segment further comprising a mutation at codon 88 and mutation(s) at codons 63 and/or 77 or a combination thereof and an indicator gene into a host cell; (b) culturing the host cell from step (a); (c) measuring the indicator in a target host cell; and (d) comparing the measurement of the indicator from step (c) with the measurement of the indicator measured when steps (a)-(c) are carried out in the absence of the candidate antiretroviral drug compound; wherein a test concentration of the candidate antiretroviral drug copound is present at steps (a)-(c); at steps (b)-(c); or at step (c).

15. A method for evaluating the biological effectiveness of a candidate **HIV** antiretroviral drug compound comprising: (a) introducing a

resistance test vector comprising a patient derived segment further comprising a mutation at codon 88 and mutation(s) at codons 63, 77, and/or 46 or a combination thereof and an indicator gene into a host cell; (b) culturing the host cell from step (a); (c) measuring the indicator in a target host cell; and (d) comparing the measurement of the indicator from step (c) with the measurement of the indicator measured when steps (a)-(c) are carried out in the absence of the candidate antiretroviral drug compound; wherein a test concentration of the candidate antiretroviral drug compound is present at steps (a)-(c); at steps (b)-(c); or at step (c).

16. A method for evaluating the biological effectiveness of a candidate **HIV** antiretroviral drug compound comprising: (a) introducing a resistance test vector comprising a patient-derived segment further comprising a mutation at codon 88 and mutation(s) at codons 63, 77, 46, 10, 20, and/or 36 or a combination thereof and an indicator gene into a host cell; (b) culturing the host cell from step (a); (c) measuring the indicator in a target host cell; and (d) comparing the measurement of the indicator from step (c) with the measurement of the indicator measured when steps (a)-(c) are carried out in the absence of the candidate antiretroviral drug compound; wherein a test concentration of the candidate antiretroviral drug compound is present at steps (a)-(c); at steps (b)-(c); or at step (c).

17. A resistance test vector comprising an **HIV**--patient-derived segment further comprising **protease** having a mutation at codon 88 and an indicator gene, wherein the expression of the indicator gene is dependent upon the patient derived segment.

18. The resistance test vector of claim 17, wherein the patient-derived segment having a mutation at codon 88 further comprises mutations at codons 63 and 77 or a combination thereof.

19. The resistance test vector of claim 17, wherein the patient-derived segment having a mutation at codon 88 further comprises mutations at codons 63, 77 and/or 46 or a combination thereof.

20. The resistance test vector of claim 17, wherein the patient-derived segment having a mutation at codon 88 further comprises mutations at codons 63, 77, 46, 10, 20 and/or 36 or a combination thereof.

21. A method for evaluating the viral fitness of a patient's virus comprising: (a) introducing a resistance test vector comprising a patient-derived segment from a patient's virus and an indicator gene into a host cell; (b) culturing the host cell from step (a); (c) measuring the luciferase activity in a target host cell in the absence of any antiretroviral drug; and (d) comparing the measurement of the indicator from step (c) with the measurement of the indicator measured when steps (a)-(c) are carried out for a reference control in the absence of any antiretroviral drug; wherein a reduction in the luciferase activity measured in step (c) as compared to step (d) indicates a reduction in viral fitness.

22. A method of assessing the effectiveness of **protease** antiretroviral therapy of an **HIV**-infected patient comprising: (a) collecting a plasma sample from the **HIV**-infected patient; (b) evaluating whether the plasma sample contains nucleic acid encoding **HIV protease** having a mutation at codon 82 and secondary positions; and (c) determining changes in susceptibility to ritonavir, nelfinavir, indinavir, saquinivir and amprenavir.

23. The method of claim 22, wherein the mutation at codon 82 codes for alanine (A), phenylalanine (F), serine (S), or threonine (T).

24. The method of claim 22, wherein the **HIV**-infected patient is being treated with an antiretroviral agent.

25. A method of assessing the effectiveness of **protease** antiretroviral therapy of an **HIV**-infected patient comprising: (a) collecting a plasma sample from the **HIV**-infected patient; (b) evaluating whether the plasma sample contains nucleic acid encoding **HIV protease** having a mutation at codon 82 and an additional mutation at codon 24; and (c) determining decreased susceptibilty to indinavir.

26. The method of claim 25, wherein the mutation at codon 24 codes for an isoleucine (I).

27. The method of claim 25, wherein the **HIV**-infected patient is being treated with an antiretroviral agent.

28. A method of assessing the effectiveness of **protease** antiretroviral therapy of an **HIV**-infected patient comprising: (a) collecting a plasma sample from the **HIV**-infected patient; (b) evaluating whether

the plasma sample contains nucleic acid encoding **HIV protease** having a mutation at codon 82 and an additional mutation at codon 71; and (c) determining decreased susceptibilty to indinavir.

29. The method of claim 28, wherein the mutation at codon 71 codes for an amino acid selected from the group consisting of a threonine, (T) valine, (V) leucine (L) and isoleucine (I).

30. The method of claim 28, wherein the **HIV**-infected patient is being treated with an antiretroviral agent.

31. A method of assessing the effectiveness of **protease** antiretroviral therapy of an **HIV**-infected patient comprising: (a) collecting a plasma sample from the **HIV**-infected patient; (b) evaluating whether the plasma sample contains nucleic acid encoding **HIV protease** having a mutation at codon 82 and additional mutations at codons selected from the group consisting of codon 54, 46, 10, 63, and a combination thereof; and (c) determining decreased susceptibilty to indinavir.

32. The method of claim 31, wherein the mutation at codon 54 codes for an amino acid selected from the group consisting of a valine (V), alanine (A), leucine (L) and threonine (T), the mutation at codon 46 codes for an amino acid selected from the group consisting of a leucine (L), isoleucine (I) and valine (V), the mutation at codon 10 codes for an amino acid selected from the group consisting of an isoleucine (I), valine (V), phenylalanine (F), and arginine (R), and the mutation at codon 63 codes for an amino acid selected from the group consisting of proline (P), alanine (A), serine (S), threonine (T), glutamine(Q),, cysteine (C), and valine (V).

33. The method of claim 31, wherein the **HIV**-infected patient is being treated with an antiretroviral agent.

34. A method of assessing the effectiveness of **protease** antiretroviral therapy of an **HIV**-infected patient comprising: (a) collecting a plasma sample from the **HIV**-infected patient; (b) evaluating whether the plasma sample contains nucleic acid encoding **HIV protease** having a mutation at codon 82 and an additional mutation at codon 20; and (c) determining decreased susceptibilty to saquinavir.

35. The method of claim 34, wherein the mutation at codon 20 codes for an amino acid selected from the group consisting of a methionine (M), threonine (T), isoleucine (I), and arginine (R).

36. The method of claim 34, wherein the **HIV**-infected patient is being treated with an antiretroviral agent.

37. A method of assessing the effectiveness of **protease** antiretroviral therapy of an **HIV**-infected patient comprising: (a) collecting a plasma sample from the **HIV**-infected patient; (b) evaluating whether the plasma sample contains nucleic acid encoding **HIV protease** having a mutation at codon 82 and an additional mutation at codon 36; and (c) determining decreased susceptibilty to saquinavir.

38. The method of claim 37, wherein the mutation at codon 36 for an amino acid selected from the group consisting of a isoleucine (I), leucine (L), and valine (V).

39. The method of claim 37, wherein the **HIV**-infected patient is being treated with an antiretroviral agent.

40. A method of assessing the effectiveness of **protease** antiretroviral therapy of an **HIV**-infected patient comprising: (a) collecting a plasma sample from the **HIV**-infected patient; (b) evaluating whether the plasma sample contains nucleic acid encoding **HIV protease** having a mutation at codon 82 and additional mutations at codons 24, 71, 54, and/or 10 or a combination thereof; and (c) determining decreased susceptibilty to saquinavir.

41. The method of claim 40, wherein the mutation at codon 24 codes for an isoleucine (I), the mutation at codon 71 codes for an amino acid selected from the group consisting of a threonine (T), valine (V), leucine (L), and isoleucine (I), the mutation at codon 54 codes for an amino acid selected from the group consisting of valine (V), alanine (A), leucine (L), and threonine (T), and the mutation at codon 10 codes for an amino acid selected from the group consisting of an isoleucine (I), valine (V), phenylalanine (F), and arginine(R).

42. The method of claim 40, wherein the **HIV**-infected patient is being treated with an antiretroviral agent.

43. A method of assessing the effectiveness of **protease** antiretroviral therapy of an **HIV**-infected patient comprising: (a) collecting a

plasma sample from the HIV-infected patient; (b) evaluating whether the plasma sample contains nucleic acid encoding **HIV protease** having a mutation at codon 82 and the number of additional mutations at secondary positions; and (c) determining decreased susceptibilty to indinavir and saquinavir.

44. The method of claim 43, wherein the number of additional mutations at secondary positions is at least 3.

45. A method of assessing the effectiveness of **protease** antiretroviral therapy of an **HIV**-infected patient comprising: (ss) collecting a plasma sample from the **HIV**-infected patient; (b) evaluating whether the plasma sample contains nucleic acid encoding **HIV protease** having a mutation at codon 90 and secondary mutations; and (c) determining changes in susceptibility to ritonavir, nelfinavir, indinavir, saquinivir and amprenavir.

46. The method of claim 45, wherein the mutation at codon 90 codes for a methionine.

47. The method of claim 45, wherein the **HIV**-infected patient is being treated with an antiretroviral agent.

48. A method of assessing the effectiveness of **protease** antiretroviral therapy of an **HIV**-infected patient comprising: (d) collecting a plasma sample from the **HIV**-infected patient; (b) evaluating whether the plasma sample contains nucleic acid encoding **HIV protease** having a mutation at codon 90 and an additional mutation at codon 73; and (c) determining decreased susceptibilty to indinavir.

49. The method of claim 48, wherein the mutation at codon 73 codes for an amino acid selected from the group consisting of a serine (S), threonine (T), and cysteine (C).

50. The method of claim 48, wherein the **HIV**-infected patient is being treated with an antiretroviral agent.

51. A method of assessing the effectiveness of **protease** antiretroviral therapy of an **HIV**-infected patient comprising: (d) collecting a plasma sample from the **HIV**-infected patient; (b) evaluating whether the plasma sample contains nucleic acid encoding **HIV protease** having a mutation at codon 90 and an additional mutation at codon 71; and (c) determining decreased susceptibilty to indinavir.

52. The method of claim 51, wherein the mutation at codon 71 codes for an amino acid selected from the group consisting of a threonine (T), valine (V), leucine (L), and isoleucine (I).

53. The method of claim 51, wherein the **HIV**-infected patient is being treated with an antiretroviral agent.

54. A method of assessing the effectiveness of **protease** antiretroviral therapy of an **HIV**-infected patient comprising: (d) collecting a plasma sample from the **HIV**-infected patient; (b) evaluating whether the plasma sample contains nucleic acid encoding **HIV protease** having a mutation at codon 90 and an additional mutation at codon 46,; and (c) determining decreased susceptibilty to indinavir.

55. The method of claim 54, wherein the mutation at codon 46 codes for an amino acid selected from the group consisting of a leucine (L), isoleucine (I) and valine (V).

56. The method of claim 54, wherein the **HIV**-infected patient is being treated with an antiretroviral agent.

57. A method of assessing the effectiveness of **protease** antiretroviral therapy of an **HIV**-infected patient comprising: (d) collecting a plasma sample from the **HIV**-infected patient; (b) evaluating whether the plasma sample contains nucleic acid encoding **HIV protease** having a mutation at codon 90 and an additional mutation at codon 73; and (c) determining decreased susceptibilty to saquinavir.

58. The method of claim 57, wherein the mutation at codon 73 codes for an amino acid selected from the group consisting of a serine (S), threonine (T), and cysteine (C).

59. The method of claim 57, wherein the **HIV**-infected patient is being treated with an antiretroviral agent.

60. A method of assessing the effectiveness of **protease** antiretroviral therapy of an **HIV**-infected patient comprising: (d) collecting a plasma sample from the **HIV**-infected patient; (b) evaluating whether the plasma sample contains nucleic acid encoding **HIV protease** having

a mutation at codon 90 and an additional mutation at codon 71, and (c) determining decreased susceptibilty to saquinavir.

61. The method of claim 60, wherein the mutation at codon 71 codes for an amino acid selected from the group consisting of a threonine (T), valine (V), leucine (L), and isoleucine (I).

62. The method of claim 60, wherein the **HIV**-infected patient is being treated with an antiretroviral agent.

63. A method of assessing the effectiveness of **protease** antiretroviral therapy of an **HIV**-infected patient comprising: (d) collecting a plasma sample from the **HIV**-infected patient; (b) evaluating whether the plasma sample contains nucleic acid encoding **HIV protease** having a mutation at codon 90 and additional mutations at codons 77 and 10; and (c) determining decreased susceptibilty to saquinavir.

64. The method of claim 63, wherein the mutation at codon 77 codes for an amino acid selected from the group consisting of isoleucine (I) and threonine (T) and the mutation at codon 10 codes for an amino acid selected from the group consisting of isoleucine (I), valine (V), phenylalanine (F), and arginine (R).

65. The method of claim 63, wherein the **HIV**-infected patient is being treated with an antiretroviral agent.

66. A method of assessing the effectiveness of **protease** antiretroviral therapy of an **HIV**-infected patient comprising: (d) collecting a plasma sample from the **HIV**-infected patient; (b) evaluating whether the plasma sample contains nucleic acid encoding **HIV protease** having a mutation at codon 90 and the number of additional mutations at secondary positions; and (c) determining decreased susceptibilty to indinavir and saquinavir.

67. The method of claim 66, wherein the number of additional mutations at secondary positions is at least 3.

68. A method of assessing the effectiveness of **protease** antiretroviral therapy of an **HIV**-infected patient comprising: (a) collecting a plasma sample from the **HIV**-infected patient; (b) evaluating whether the plasma sample contains nucleic acid encoding **HIV protease** having a mutation at codons 82 and 90 and secondary mutations; and (c) determining changes in susceptibility to ritonavir, nelfinavir, indinavir, saquinivir and amprenavir.

69. The method of claim 68, wherein the mutation at codon 82 codes for an amino acid selected from the group consisting of alanine (A), phenylalanine (F), serine (S), and threonine (T) and the mutation at codon 90 codes for a methionine (M).

70. The method of claim 68, wherein the **HIV**-infected patient is being treated with an antiretroviral agent.

71. A method for evaluating the biological effectiveness of a candidate **HIV protease** antiretroviral drug compound comprising: (a) introducing a resistance test vector comprising a patient-derived segment further comprising a mutation at codon 82 and additional mutations at one or more secondary positions and an indicator gene into a host cell; (b) culturing the host cell from step (a); (c) measuring the indicator in a target host cell; and (d) comparing the measurement of the indicator from step (c) with the measurement of the indicator measured when steps (a)-(c) are carried out in the absence of the candidate antiretroviral drug compound; wherein a test concentration of the candidate antiretroviral drug compound is present at steps (a)-(c); at steps (b)-(c); or at step (c).

72. A method for evaluating the biological effectiveness of a candidate **HIV protease** antiretroviral drug compound comprising: (a) introducing a resistance test vector comprising a patient-derived segment further comprising a mutation at codon 82 and secondary mutation(s) at codons 20, 24, 71, 54 and/or 10 or a combination thereof and an indicator gene into a host cell; (b) culturing the host cell from step (a); (c) measuring the indicator in a target host cell; and (d) comparing the measurement of the indicator from step (c) with the measurement of the indicator measured when steps (a)-(c) are carried out in the absence of the candidate antiretroviral drug compound; wherein a test concentration of the candidate antiretroviral drug compound is present at steps (a)-(c); at steps (b)-(c); or at step (c).

73. A method for evaluating the biological effectiveness of a candidate **HIV protease** antiretroviral drug compound comprising: (a) introducing a resistance test vector comprising a patient-derived segment further comprising a mutation at codon 90 and additional

mutations at one or more secondary positions and an indicator gene into a host cell; (b) culturing the host cell from step (a); (c) measuring the indicator in a target host cell; and (d) comparing the measurement of the indicator from step (c) with the measurement of the indicator measured when steps (a)-(c) are carried out in the absence of the candidate antiretroviral drug compound; wherein a test concentration of the candidate antiretroviral drug compound is present at steps (a)-(c); at steps (b)-(c); or at step (c).

74. A method for evaluating the biological effectiveness of a candidate **HIV protease** antiretroviral drug compound comprising: (a) introducing a resistance test vector comprising a patient-derived segment further comprising a mutation at codon 90 and secondary mutation(s) at codons 73, 71, 10 and/or 46 or a combination thereof and an indicator gene into a host cell; (b) culturing the host cell from step (a); (c) measuring the indicator in a target host cell; and (d) comparing the measurement of the indicator from step (c) with the measurement of the indicator measured when steps (a)-(c) are carried out in the absence of the candidate antiretroviral drug compound; wherein a test concentration of the candidate antiretroviral drug compound is present at steps (a)-(c); at steps (b)-(c); or at step (c).

75. A method for evaluating the biological effectiveness of a candidate **HIV protease** antiretroviral drug compound comprising: (a) introducing a resistance test vector comprising a patient-derived segment further comprising a mutation at codons 82 and 90 and additional mutations at one or more secondary positions and an indicator gene into a host cell; (b) culturing the host cell from step (a); (c) measuring the indicator in a target host cell; and (d) comparing the measurement of the indicator from step (c) with the measurement of the indicator measured when steps (a)-(c) are carried out in the absence of the candidate antiretroviral drug compound; wherein a test concentration of the candidate antiretroviral drug compound is present at steps (a)-(c); at steps (b)-(c); or at step (c).

76. A resistance test vector comprising an **HIV** patient-derived segment further comprising **protease** having a mutation at codon 82 and an indicator gene, wherein the expression of the indicator gene is dependent upon the patient derived segment.

77. The resistance test vector of claim 76, wherein the patient-derived segment having a mutation at codon 82 further comprises at least one secondary mutation at a codon selected from the group consisting of 20, 24, 71, 54, 10 and a combination thereof.

78. The resistance test vector of claim 76, wherein the patient-derived segment having a mutation at codon 90 further comprises at least one secondary mutation at a codon selected from the group consisting of 73, 71, 46, 10 and a combination thereof.

79. A method for determining replication capacity for a patient's virus comprising: (a) introducing a resistance test vector comprising a patient derived segment and an indicator gene into a host cell; (b) culturing the host cell from (a); (c) harvesting viral particles from step (b) and infecting target host cells; (d) measuring expression of the indicator gene in the target host cell, wherein the expression of the indicator gene is dependent upon the patient-derived segment; (e) comparing the expression of the indicator gene from (d) with the expression of the indicator gene measured when steps (a) through (d) are carried out in a control resistance test vector; and (f) normalizing the expression of the indicator gene by measuring an amount of virus in step (c).

80. A method of assessing the effectiveness of **protease** antiretroviral therapy of an **HIV**-infected patient comprising: (a) collecting a biological sample from the **HIV**-infected patient; (b) evaluating whether the biological sample contains nucleic acid encoding **HIV protease** having a mutation at codon 82 or codon 90; and (c) determining changes in susceptibility to **protease** inhibitors.

81. The method of claim 80, wherein step (c) determines changes in susceptibility to saquinavir.

82. The method of claim 80, wherein the mutation at codon 82 codes for alanine (A), phenylalanine (F), serine (S), or threonine (T).

83. The method of claim 82, wherein the mutation at codon 82 is a substitution of alanine (A), phenylalanine (F), serine (S), or threonine (T) for valine(V).

84. The method of claim 80, wherein the mutation at codon 90 codes for methionine (M).

85. The method of claim 84, wherein the mutation at codon 90 is a substitution of methionine (M) for leucine (L).

86. A method for evaluating the biological effectiveness of a candidate **HIV protease** antiretroviral drug compound comprising: (a) introducing a resistance test vector comprising a patient-derived segment having nucleic acid encoding **HIV protease** with a mutation at codon 82 or codon 90 and an indicator gene into a host cell; (b) culturing the host cell from step (a); (c) measuring the indicator gene in a target host cell; and (d) comparing the measurement of the indicator gene from step (c) with the measurement of the indicator gene measured when steps (a)-(c) are carried out in the absence of the candidate antiretroviral drug compound; wherein a test concentration of the candidate antiretroviral drug compound is present at steps (a)-(c); at steps (b)-(c); or at step (c).

87. A resistance test vector comprising an **HIV** patient-derived segment further comprising **protease** having a mutation at codon 82 or codon 90 and an indicator gene, wherein the expression of the indicator gene is dependent upon the patient-derived segment.

88. The resistance test vector of claim 87, wherein the patient-derived segment having a mutation at codon 82 codes for alanine (A), phenylalanine (F), serine (S), or threonine (T).

89. The resistance test vector of claim 88, wherein the patient-derived segment having a mutation at codon 82 is a substitution of alanine (A), phenylalanine (F), serine (S), or threonine (T) for valine(V).

90. The resistance test vector of claim 87, wherein the patient-derived segment having a mutation at codon 90 codes for methionine (M).

91. The resistance test vector of claim 90, wherein the patient-derived segment having a mutation at codon 90 is a substitution of methionine (M) for leucine (L).

92. A method for determining replication capacity for a patient's virus comprising: (a) introducing a resistance test vector comprising a patient-derived segment and an indicator gene into a host cell; (b) culturing the host cell from (a); (c) harvesting viral particles from step (b) and infecting target host cells; (d) measuring expression of the indicator gene in the target host cell, wherein the expression of the indicator gene is dependent upon the patient-derived segment; and (e) comparing the expression of the indicator gene from (d) with the expression of the indicator gene measured when steps (a) through (d) are carried out in a control resistance test vector.

93. The method of claim 92 further comprising the step of: (f) normalizing the expression of the indicator gene by measuring an amount of virus in step (c).

94. The method of claim 92 wherein the patient-derived segment comprises nucleic acid encoding **HIV** integrase having a mutation at codon 66.

95. The method of claim 92 wherein the patient-derived segment comprises nucleic acid encoding **HIV** integrase having a mutation at codon 154.

96. The method of claim 94 wherein the patient-derived segment comprises nucleic acid encoding **HIV** integrase having an additional mutation at codon 153.

97. The method of claim 94 wherein the patient-derived segment comprises nucleic acid encoding **HIV** integrase having an additional mutation at codon 154.

98. A method of assessing the effectiveness of **protease** antiretroviral therapy of an **HIV**-infected patient comprising: (uuuu) collecting a biological sample from the **HIV**-infected patient; (b) evaluating whether the biological sample contains nucleic acid encoding **HIV protease** having a mutation at codon 82 and a secondary mutation at codons selected from the group consisting of 73, 55, 48, 20, 43, 53, 90, 13, 84, 23, 33, 74, 32, 39, 60, 36, and 35, or a mutation at codon 90 and a secondary mutation at codons selected from the group consisting of 53, 95, 54, 84, 82, 46, 13, 74, 55, 85, 20, 72, 62, 66, 84, 48, 33, 73, 71, 64, 93, 23, 58, and 36; and (c) determining a change in susceptibility to a **protease** inhibitor.

99. The method of claim 98, wherein the mutation at codon 82 is a substitution of alanine (A), phenylalanine (F), serine (S), or threonine (T) for valine(V) and the mutation at codon 90 is a substitution of methionine (M) for leucine (L).

100. The method of claim 99, wherein the **protease** inhibitor is

selected from the group consisting of indinavir, amprenavir, and saquinavir.

101. The method of claim 100, having a mutation at codon 82 and a secondary mutation at codons selected from the group consisting of 84, 48, 23, 73, 53, 33, 74, 20, 90, 32 and 39 or a mutation at codon 90 and a secondary mutation at codons selected from the group consisting of 53, 66, 84, 54, 48, 33, 73, 20, 71, 64 and 93, wherein the **protease** inhibitor is saquinavir.

102. The method of claim 101, having a mutation at codon 82 and a secondary mutation at codons selected from the group consisting of 84, 48, 23, 73, 53, 33, 74, 20, and 90, or a mutation at codon 90 and a secondary mutation at codons selected from the group consisting of 53, 66, 84, 54, 48, 33, 73, 20, and 71, wherein the change in susceptibility in step (c) is a decrease in susceptibility to saquinavir.

103. The method of claim 101, having a mutation at codon 82 and a secondary mutation at codons 32 or 39, or a mutation at codon 90 and a secondary mutation at codons 64 or 93, wherein the change in susceptibility in step (c) is an increase in susceptibility to saquinavir.

104. The method of claim 100, having a mutation at codon 90 and a secondary mutation at codons selected from the group consisting of 53, 95, 54, 84, 82, 46, 13, and 74, wherein the **protease** inhibitor is indinavir.

105. The method of claim 104, having a mutation at codon 90 and a secondary mutation at codons selected from the group consisting of 53, 95, 54, 84, 82, and 46, wherein the change in susceptibility in step (c) is a decrease in susceptibility to indinavir.

106. The method of claim 104, having a mutation at codon 90 and a secondary mutation at codons 13 or 74, wherein the change in susceptibility in step (c) is an increase in susceptibility to indinavir.

107. The method of claim 100, having a mutation at codon 82 and a secondary mutation at codons selected from the group consisting of 73, 55, 48, 20, 43, 53, 90, 13, 48, 23, 84, 53, 74, 60, 33, 36, 35, 32, and 46 or a mutation at codon 90 and a secondary mutation at codons selected from the group consisting of 95, 55, 54, 82, 85, 84, 20, 72, 62, 74, 53, 48, 23, 58, 36, 64, 77, and 93.

108. The method of claim 107, wherein the **protease** inhibitor is selected from the group consisting of indinavir, amprenavir, and saquinavir.

109. The method of claim 108, wherein step (c) is determining a change in susceptibility to the **protease** inhibitor greater than 10 fold.

110. The method of claim 108, having a mutation at codon 82 and a secondary mutation at codons selected from the group consisting of 48, 23, 84, 53, 74, 20, 60, 33, 36, 35, or a mutation at codon 90 and a secondary mutation at codons selected from the group consisting of 84, 53, 48, 23, 58, 20, 36, and 54, wherein the change in susceptibility in step (c) is a decrease in susceptibility to saquinavir.

111. The method of claim 108, having a mutation at codon 82 and a secondary mutation at codons 32 or 46, or a mutation at codon 90 and a secondary mutation at codons 64, 77, or 93, wherein the change in susceptibility in step (c) is an increase in susceptibility to saquinavir.

112. The method of claim 108, having a mutation at codon 82 and a secondary mutation at codons selected from the group consisting of 73, 55, 48, 20, 43, 53, and 90, or a mutation at codon 90 and a secondary mutation at codons selected from the group consisting of 95, 55, 54, 82, 85, 84, 20, 72, and 62, wherein the change in susceptibility in step (c) is a decrease in susceptibility to indinavir.

113. The method of claim 108, having a mutation at codon 82 and a secondary mutation at codon 13, or a mutation at codon 90 and a secondary mutation at codon 74, wherein the change in susceptibility in step (c) is an increase in susceptibility to indinavir.

114. A method of assessing the effectiveness of **protease** antiretroviral therapy of an **HIV**-infected patient comprising: (kkkkk) collecting a biological sample from the **HIV**-infected patient; (b) evaluating whether the biological sample contains nucleic acid encoding **HIV protease** having a mutation at codon 90 and secondary mutations of at least three codons; and (c) determining a decrease in

susceptibility to saquinavir.

115. The method of claim 114, wherein in the evaluating step (b), the nucleic acid encoding **HIV protease** has secondary mutations of at least five codons.

116. The method of claim 114, wherein the secondary mutation are selected from the group consisting of codons 10, 20, 52, 53, 54, 66, 71, 73 and 84.

117. A method of assessing the effectiveness of **protease** antiretroviral therapy of an **HIV**-infected patient comprising: (nnnnn) collecting a biological sample from the **HIV**-infected patient; (b) evaluating whether the biological sample contains nucleic acid encoding **HIV protease** having a mutation at codon 82 and secondary mutations at codons selected from the group consisting of 33, 23, 84, 32, 53, 90, 37, 71, 46, 10, 54, 61, 11, and 46, or a mutation at codon 90 and secondary mutations at codons selected from the group consisting of 89, 53, 84, 33, 92, 95, 54, 58, 46, 82, 36, 10, 62, 74, 15, 47, 66, 32, 55, 53, 13, and 69; and (c) determining a change in susceptibility to amprenavir.

118. The method of claim 117, wherein the mutation at codon 82 is a substitution of alanine (A), phenylalanine (F), serine (S), or threonine (T) for valine(V) and the mutation at codon 90 is a substitution of methionine (M) for leucine (L).

119. The method of claim 118, having a mutation at codon 82 and secondary mutations at codons selected from the group consisting of 33, 23, 84, 32, 53, 90, 37, 71, 46, 10, 54, 11, and 46, or a mutation at codon 90 and secondary mutations at codons selected from the group consisting of 89, 53, 84, 33, 92, 95, 54, 58, 46, 82, 36, 10, 62, 47, 66, 32, 55, 53, and 13; wherein the change in susceptibility in step (c) is a decrease in susceptibility to saquinavir.

120. The method of claim 118, having a mutation at codon 82 and a secondary mutation at codon 61, or a mutation at codon 90 and secondary mutations at codons 74, 15, or 69, wherein the change in susceptibility in step (c) is an increase in susceptibility to saquinavir.

121. A resistance test vector comprising an **HIV** patient-derived segment comprising nucleic acid encoding **protease** having a mutation at codon 82 and secondary mutations at codons selected from the group consisting of 73, 55, 48, 20, 43, 53, 90, 13, 84, 23, 33, 74, 32, 39, 60, 36, and 35, or a mutation at codon 90 and secondary mutations at codons selected from the group consisting of 53, 95, 54, 84, 82, 46, 13, 74, 55, 85, 20, 72, 62, 66, 84, 48, 33, 73, 71, 64, 93, 23, 58, and 36 and an indicator gene, wherein the expression of the indicator gene is dependent upon the patient-derived segment.

122. The resistance test vector of claim 121, wherein the mutation of the patient derived segment at codon 82 is a substitution of alanine (A), phenylalanine (F), serine (S), or threonine (T) for valine(V) and the mutation at codon 90 is a substitution of methionine (M) for leucine (L).

123. A method for determining whether an **HIV** virus obtained from a patient infected with **HIV** is resistant to IDV, LPV, NFV and RTV which comprises determining whether a mutation at position 30 from D to N exists in the **HIV protease** obtained from the patient, wherein the presence o the mutation indicates that the virus is resistant to IDV, LPV, NFV and RTV.

124. A method for determining whether an **HIV** virus obtained from a patient infected with **HIV** is resistant to IDV, LPV, NFV or RTV which comprises determining whether the virus is resistant to any one of IDV, LPV, NFV or RTV, wherein a determination that the virus is resistant to any one of IDV, LPV, NFV or RTV is indicative of the virus being resistant to IDV, LPV, NFV and RTV.

125. A method for determining cross resistance of an **HIV** virus to RTV and SQV which comprises determining (i) whether position 30 of the **HIV protease** is D, and (ii) whether the virus is resistant to NFV, wherein a mutation from D to N at position 30 of **HIV protease** and resistance of the virus to NFV are indicative of cross resistance to IDV and SQV.

126. A method for determining whether an **HIV** virus obtained from a patient infected with **HIV** is resistant to LPV and IND which comprises determining whether position 50 of the **HIV protease** of the virs is I or V, wherein the determination that position 50 is V is indicative of the virus being resistant to LPV and IND.

TABLE A

Summary of Replication Capacity (RC) and Enzyme Function Results

	LOW RC (<25% of Ref.*)	MEDIUM RC (26-75% of Ref.)	HIGH RC (>75% of Ref.)
% of Total Tested	41% (55)	45% (59)	14% (19)
PR Processing Defects (% p41 > 10%)	71% (39)	24% (14)	10% (2)
Impaired RT Activity (<25% of reference)	14% (7)	2% (1)	0%
>10 mutations in **Protease**	62% (34)	22% (13)	5% (1)
>10X reduced susceptibility to NFV	63% (35)	32% (19)	16% (3)

*Reference virus: NL4-3

123. A method for determining whether an **HIV** virus obtained from a patient infected with **HIV** has reduced susceptibility to IDV, NFV, SQV and RTV which comprises determining the susceptibility to one of these drugs and whether a mutation at position 30 from D to N exists in the **HIV protease** obtained from the patient, wherein the absence of the mutation indicates that the susceptibility of the virus to IDV, LPV, NFV and RTV has a high probability of being the same.

124. A method for determining whether an **HIV** virus obtained from a patient infected with **HIV** has reduced susceptibility to IDV, LPV, NFV or RTV which comprises determining whether the virus has reduced susceptibility to any one of IDV, LPV, NFV or RTV, wherein a determination that the virus has reduced susceptibility to any one of IDV, LPV, NFV or RTV is indicative of the virus having reduced susceptibility to IDV, LPV, NFV and RTV.

125. A method for determining whether an **HIV** virus obtained from a patient infected with **HIV** is resistant (>10-fold change in IC50) to LPV which comprises determining whether position 50 of the **HIV protease** of the virus is I or V, wherein the determination that position 50 is V and 2 other **protease** mutations from the list of positions associated with resistance to LPV (10, 20, 24, 46, 53, 54, 63, 71, 82, 84 and 90) is indicative of the virus being resistant to LPV.

L4 ANSWER 3 OF 3 USPATFULL on STN
2002:126326 Means and methods for monitoring protease inhibitor antiretroviral therapy and guiding therapeutic decisions in the treatment of HIV/AIDS.
Parkin, Neil T., Belmont, CA, UNITED STATES
Ziermann, Rainer A., San Mateo, CA, UNITED STATES
US 2002064838 A1 20020530
APPLICATION: US 2001-766344 A1 20010119 (9)
DOCUMENT TYPE: Utility; APPLICATION.
CAS INDEXING IS AVAILABLE FOR THIS PATENT.
CLM What is claimed is:

1. A method of assessing the effectiveness of **protease** antiretroviral therapy of an **HIV**-infected patient comprising: (a) collecting a plasma sample from the **HIV**-infected patient; (b) evaluating whether the plasma sample contains nucleic acid encoding **HIV protease** having a mutation at codon 88; and (c) determining **increased susceptibility** to amprenavir.

2. The method of claim 1, wherein the mutation at codon 88 codes for a serine (S).

3. The method of claim 1, wherein the **HIV**-infected patient is being treated with an antiretroviral agent.

4. A method of assessing the effectiveness of **protease** antiretroviral therapy of an **HIV**-infected patient comprising: (a) collecting a plasma sample from the **HIV**-infected patient; (b) evaluating whether the plasma sample contains nucleic acid encoding **HIV protease** having a mutation at codon 88 and additional mutations at codons 63 and/or 77 or a combination thereof; and (c) determining decreased susceptibilty to nelfinavir and indinavir and **increased susceptibility** to amprenavir.

5. The method of claim 4, wherein the mutation at codon 63 codes for a proline (P) or a glutamine (Q) and the mutation at codon 77 codes for an isoleucine (I).

6. The method of claim 1, wherein the **HIV**-infected patient is being treated with an antiretroviral agent.

7. A method of assessing the effectiveness of **protease** antiretroviral therapy of an **HIV**-infected patient comprising: (a) collecting a plasma sample from the **HIV**-infected patient; (b) evaluating whether the plasma sample contains nucleic acid encoding **HIV protease** having a mutation at codon 88 and additional mutations at codons 63, 77 and/or 46 or a combination thereof; and (c) determining decreased susceptibilty to nelfinavir and indinavir and **increased susceptibility** to amprenavir.

8. The method of claim 7, wherein the mutation at codon 63 codes for a proline (P) or a glutamine (Q), the mutation at codon 77 codes for an isoleucine (I).and the mutation at codon 46 codes for a leucine (L) or an isoleucine (I).

9. The method of claim 7, wherein the **HIV**-infected patient is being treated with an antiretroviral agent.

10. A method of assessing the effectiveness of **protease** antiretroviral therapy of an **HIV**-infected patient comprising: (a) collecting a plasma sample from the **HIV**-infected patient; (b) evaluating whether the plasma sample contains nucleic acid encoding **HIV protease** having a mutation at codon 88 and additional mutations at codons 63, 77, 46, 10, 20, and/or 36 or a combination thereof; and (c) determining decreased susceptibilty to nelfinavir and indinavir and **increased susceptibility** to amprenavir.

11. The method of claim 10, wherein the mutation at codon 63 codes for a proline (P) or a glutamine (Q), the mutation at codon 77 codes for an isoleucine (I), the mutation at codon 46 codes for a leucine (L) or an isoleucine (I), the mutation at codon 10 codes for a isoleucine (I) or a phenylalanine (F), the mutation at 20 codes for a threonine (T) or a methionine (M) or an arginine (R), and the mutation at 36 codes for an isoleucine (I) or a valine (V).

12. The method of claim 10, wherein the **HIV**-infected patient is being treated with an antiretroviral agent.

13. A method for evaluating the biological effectiveness of a candidate **HIV** antiretroviral drug compound comprising: (a) introducing a resistance test vector comprising a patient-derived segment further comprising a mutation at codon 88 and an indicator gene into a host cell; (b) culturing the host cell from step (a); (c) measuring the indicator in a target host cell; and (d) comparing the measurement of the indicator from step (c) with the measurement of the indicator measured when steps (a)-(c) are carried out in the absence of the candidate antiretroviral drug compound; wherein a test concentration of the candidate antiretroviral drug compound is present at steps (a)-(c); at steps (b)-(c); or at step (c).

14. A method for evaluating the biological effectiveness of a candidate **HIV** antiretroviral drug compound comprising: (a) introducing a resistance test vector comprising a patient-derived segment further comprising a mutation at codon 88 and mutation(s) at codons 63 and/or 77 or a combination thereof and an indicator gene into a host cell; (b) culturing the host cell from step (a); (c) measuring the indicator in a target host cell; and (d) comparing the measurement of the indicator from step (c) with the measurement of the indicator measured when steps (a)-(c) are carried out in the absence of the candidate antiretroviral drug compound; wherein a test concentration of the candidate antiretroviral drug copound is present at steps (a)-(c); at steps (b)-(c); or at step (c).

15. A method for evaluating the biological effectiveness of a candidate **HIV** antiretroviral drug compound comprising: (a) introducing a resistance test vector comprising a patient-derived segment further comprising a mutation at codon 88 and mutation(s) at codons 63, 77, and/or 46 or a combination thereof and an indicator gene into a host cell; (b) culturing the host cell from step (a); (c) measuring the indicator in a target host cell; and (d) comparing the measurement of the indicator from step (c) with the measurement of the indicator measured when steps (a)-(c) are carried out in the absence of the candidate antiretroviral drug compound; wherein a test concentration of the candidate antiretroviral drug compound is present at steps (a)-(c); at steps (b)-(c); or at step (c).

16. A method for evaluating the biological effectiveness of a candidate **HIV** antiretroviral drug compound comprising: (a) introducing a resistance test vector comprising a patient-derived segment further comprising a mutation at codon 88 and mutation(s) at codons 63, 77, 46, 10, 20, and/or 36 or a combination thereof and an indicator gene into a

host cell; (b) culturing the host cell from step (a); (c) measuring the indicator in a target host cell; and (d) comparing the measurement of the indicator from step (c) with the measurement of the indicator measured when steps (a)-(c) are carried out in the absence of the candidate antiretroviral drug compound; wherein a test concentration of the candidate antiretroviral drug compound is present at steps (a)-(c); at steps (b)-(c); or at step (c).

17. A resistance test vector comprising an **HIV** patient-derived segment further comprising **protease** having a mutation at codon 88 and an indicator gene, wherein the expression of the indicator gene is dependent upon the patient derived segment.

18. The resistance test vector of claim 17, wherein the patient-derived segment having a mutation at codon 88 further comprises mutations at codons 63 and 77 or a combination thereof.

19. The resistance test vector of claim 17, wherein the patient-derived segment having a mutation at codon 88 further comprises mutations at codons 63, 77 and/or 46 or a combination thereof.

20. The resistance test vector of claim 17, wherein the patient-derived segment having a mutation at codon 88 further comprises mutations at codons 63, 77, 46, 10, 20 and/or 36 or a combination thereof.

21. A method for evaluating the viral fitness of a patient's virus comprising: (a) introducing a resistance test vector comprising a patient-derived segment from a patient' s virus and an indicator gene into a host cell; (b) culturing the host cell from step (a); (c) measuring the luciferase activity in a target host cell in the absence of any antiretroviral drug; and (d) comparing the measurement of the indicator from step (c) with the measurement of the indicator measured when steps (a)-(c) are carried out for a reference control in the absence of any antiretroviral drug; wherein a reduction in the luciferase activity measured in step (c) as compared to step (d) indicates a reduction in viral fitness.

22. A method of assessing the effectiveness of **protease** antiretroviral therapy of an **HIV**-infected patient comprising: (a) collecting a plasma sample from the **HIV**-infected patient; (b) evaluating whether the plasma sample contains nucleic acid encoding **HIV protease** having a mutation at codon 82 and secondary positions; and (c) determining changes in susceptibility to ritonavir, nelfinavir, indinavir, saquinivir and amprenavir.

23. The method of claim 22, wherein the mutation at codon 82 codes for alanine (A), phenylalanine (F), serine (S), or threonine (T).

24. The method of claim 22, wherein the **HIV**-infected patient is being treated with an antiretroviral agent.

25. A method of assessing the effectiveness of **protease** antiretroviral therapy of an **HIV**-infected patient comprising: (a) collecting a plasma sample from the **HIV**-infected patient; (b) evaluating whether the plasma sample contains nucleic acid encoding **HIV protease** having a mutation at codon 82 and an additional mutation at codon 24; and (c) determining decreased susceptibilty to indinavir.

26. The method of claim 25, wherein the mutation at codon 24 codes for an isoleucine (I).

27. The method of claim 25, wherein the **HIV**-infected patient is being treated with an antiretroviral agent.

28. A method of assessing the effectiveness of **protease** antiretroviral therapy of an **HIV**-infected patient comprising: (a) collecting a plasma sample from the **HIV**-infected patient; (b) evaluating Whether the plasma sample contains nucleic acid encoding **HIV protease** having a mutation at codon 82 and an additional mutation at codon 71; and (c) determining decreased susceptibilty to indinavir.

29. The method of claim 28, wherein the mutation at codon 71 codes for an amino acid selected from the group consisting of a threonine, (T) valine, (V) leucine (L) and isoleucine (I).

30. The method of claim 28, wherein the **HIV**-infected patient is being treated with an antiretroviral agent.

31. A method of assessing the effectiveness of **protease** antiretroviral therapy of an **HIV**-infected patient comprising: (a) collecting a plasma sample from the **HIV**-infected patient; (b) evaluating whether the plasma sample contains nucleic acid encoding **HIV protease** having a mutation at codon 82 and additional mutations at codons selected from

the group consisting of codon 54, 46, 10, 63, and a combination thereof, and (c) determining decreased susceptibilty to indinavir.

32. The method of claim 31, wherein the mutation at codon 54 codes for an amino acid selected from the group consisting of a valine (V), alanine (A), leucine (L) and threonine (T), the mutation at codon 46 codes for an amino acid selected from the group consisting of a leucine (L), isoleucine (I) and valine (V), the mutation at codon 10 codes for an amino acid selected from the group consisting of an isoleucine (I), valine (V), phenylalanine (F), and arginine (R), and the mutation at codon 63 codes for an amino acid selected from the group consisting of proline (P), alanine (A), serine (S), threonine (T), glutamine(Q), cysteine (C), and valine (V).

33. The method of claim 31, wherein the **HIV**-infected patient is being treated with an antiretroviral agent.

34. A method of assessing the effectiveness of **protease** antiretroviral therapy of an **HIV**-infected patient comprising: (a) collecting a plasma sample from the **HIV**-infected patient; (b) evaluating whether the plasma sample contains nucleic acid encoding **HIV protease** having a mutation at codon 82 and an additional mutation at codon 20; and ( ) determining decreased susceptibilty to saquinavir.

35. The method of claim 34, wherein the mutation at codon 20 codes for an amino acid selected from the group consisting of a methionine (M), threonine (T), isoleucine (I), and arginine (R).

36. The method of claim 34, wherein the **HIV**-infected patient is being treated with an antiretroviral agent.

37. A method of assessing the effectiveness of **protease** antiretroviral therapy of an **HIV**-infected patient comprising: (a) collecting a plasma sample from the **HIV**-infected patient; (b) evaluating whether the plasma sample contains nucleic acid encoding **HIV protease** having a mutation at codon 82 and an additional mutation at codon 36; and ( ) determining decreased susceptibilty to saquinavir.

38. The method of claim 37, wherein the mutation at codon 36 for an amino acid selected from the group consisting of a isoleucine (I), leucine (L), and valine (V).

39. The method of claim 37, wherein the **HIV**-infected patient is being treated with an antiretroviral agent.

40. A method of assessing the effectiveness of **protease** antiretroviral therapy of an **HIV**-infected patient comprising: (a) collecting a plasma sample from the **HIV**-infected patient; (b) evaluating whether the plasma sample contains nucleic acid encoding **HIV protease** having a mutation at codon 82 and additional mutations at codons 24, 71, 54, and/or 10 or a combination thereof; and (c) determining decreased susceptibilty to saquinavir.

41. The method of claim 40, wherein the mutation at codon 24 codes for an isoleucine (I), the mutation at codon 71 codes for an amino acid selected from the group consisting of a threonine (T), valine (V), leucine (L), and isoleucine (I), the mutation at codon 54 codes for an amino acid selected from the group consisting of valine (V), alanine (A), leucine (L), and threonine (T), and the mutation at codon 10 codes for an amino acid selected from the group consisting of an isoleucine (I), valine (V), phenylalanine (F), and arginine(R).

42. The method of claim 40, wherein the **HIV**-infected patient is being treated with an antiretroviral agent.

43. A method of assessing the effectiveness of **protease** antiretroviral therapy of an **HIV**-infected patient comprising: (a) collecting a plasma sample from the **HIV**-infected patient; (b) evaluating whether the plasma sample contains nucleic acid encoding **HIV protease** having a mutation at codon 82 and the number of additional mutations at secondary positions; and (c) determining decreased susceptibilty to indinavir and saquinavir.

44. The method of claim 43, wherein the number of additional mutations at secondary positions is at least 3.

45. A method of assessing the effectiveness of **protease** antiretroviral therapy of an **HIV**-infected patient comprising: (a) collecting a plasma sample from the **HIV**-infected patient; (b) evaluating whether the plasma sample contains nucleic acid encoding **HIV protease** having a mutation at codon 90 and secondary mutations; and (c) determining changes in susceptibility to ritonavir, nelfinavir, indinavir, saquinivir and amprenavir.

46. The method of claim 45, wherein the mutation at codon 90 codes for a methionine.

47. The method of claim 45, wherein the **HIV**-infected patient is being treated with an antiretroviral agent.

48. A method of assessing the effectiveness of **protease** antiretroviral therapy of an **HIV**-infected patient comprising: (a) collecting a plasma sample from the **HIV**-infected patient; (b) evaluating whether the plasma sample contains nucleic acid encoding **HIV protease** having a mutation at codon 90 and an additional mutation at codon 73; and (c) determining decreased susceptibilty to indinavir.

49. The method of claim 48, wherein the mutation at codon 73 codes for an amino acid selected from the group consisting of a serine (S), threonine (T), and cysteine (C)

50. The method of claim 48, wherein the **HIV**-infected patient is being treated with an antiretroviral agent.

51. A method of assessing the effectiveness of **protease** antiretroviral therapy of an **HIV**-infected patient comprising: (a) collecting a plasma sample from the **HIV**-infected patient; (b) evaluating whether the plasma sample contains nucleic acid encoding **HIV protease** having a mutation at codon 90 and an additional mutation at codon 71; and (c) determining decreased susceptibilty to indinavir.

52. The method of claim 51, wherein the mutation at codon 71 codes for an amino acid selected from the group consisting of a threonine (T), valine (V), leucine (L), and isoleucine (I).

53. The method of claim 51, wherein the **HIV**-infected patient is being treated with an antiretroviral agent.

54. A method of assessing the effectiveness of **protease** antiretroviral therapy of an **HIV**-infected patient comprising: (a) collecting a plasma sample from the **HIV**-infected patient; (b) evaluating whether the plasma sample contains nucleic acid encoding **HIV protease** having a mutation at codon 90 and an additional mutation at codon 46,; and (c) determining decreased susceptibilty to indinavir.

55. The method of claim 54, wherein the mutation at codon 46 codes for an amino acid selected from the group consisting of a leucine (L), isoleucine (I) and valine (V).

56. The method of claim 54, wherein the **HIV**-infected patient is being treated with an antiretroviral agent.

57. A method of assessing the effectiveness of **protease** antiretroviral therapy of an **HIV**-infected patient comprising: (a) collecting a plasma sample from the **HIV**-infected patient; (b) evaluating whether the plasma sample contains nucleic acid encoding **HIV protease** having a mutation at codon 90 and an additional mutation at codon 73; and (c) determining decreased susceptibilty to saquinavir.

58. The method of claim 57, wherein the mutation at codon 73 codes for an amino acid selected from the group consisting of a serine (S), threonine (T), and cysteine (C).

59. The method of claim 57, wherein the **HIV**-infected patient is being treated with an antiretroviral agent.

60. A method of assessing the effectiveness of **protease** antiretroviral therapy of an **HIV**-infected patient comprising: (a) collecting a plasma sample from the **HIV**-infected patient; (b) evaluating whether the plasma sample contains nucleic acid encoding **HIV protease** having a mutation at codon 90 and an additional mutation at codon 71; and (c) determining decreased susceptibilty to saquinavir.

61. The method of claim 60, wherein the mutation at codon 71 codes for an amino acid selected from the group consisting of a threonine (T), valine (V), leucine (L), and isoleucine (I).

62. The method of claim 60, wherein the **HIV**-infected patient is being treated with an antiretroviral agent.

63. A method of assessing the effectiveness of **protease** antiretroviral therapy of an **HIV**-infected patient comprising: (a) collecting a plasma sample from the **HIV**-infected patient; (b) evaluating whether the plasma sample contains nucleic acid encoding **HIV protease** having a mutation at codon 90 and additional mutations at codons 77 and 10; and ( ) determining decreased susceptibilty to saquinavir.

64. The method of claim 63, wherein the mutation at codon 77 codes for an amino acid selected from the group consisting of isoleucine (I) and threonine (T) and the mutation at codon 10 codes for an amino acid selected from the group consisting of isoleucine (I), valine (V), phenylalanine (F), and arginine (R).

65. The method of claim 63, wherein the **HIV**-infected patient is being treated with an antiretroviral agent.

66. A method of assessing the effectiveness of **protease** antiretroviral therapy of an **HIV**-infected patient comprising: (a) collecting a plasma sample from the **HIV**-infected patient; (b) evaluating whether the plasma sample contains nucleic acid encoding **HIV protease** having a mutation at codon 90 and the number of additional mutations at secondary positions; and (c) determining decreased susceptibilty to indinavir and saquinavir.

67. The method of claim 66, wherein the number of additional mutations at secondary positions is at least 3.

68. A method of assessing the effectiveness of **protease** antiretroviral therapy of an **HIV**-infected patient comprising: (a) collecting a plasma sample from the **HIV**-infected patient; (b) evaluating whether the plasma sample contains nucleic acid encoding **HIV protease** having a mutation at codons 82 and 90 and secondary mutations; and (c) determining changes in susceptibility to ritonavir, nelfinavir, indinavir, saquinivir and amprenavir.

69. The method of claim 68, wherein the mutation at codon 82 codes for an amino acid selected from the group consisting of alanine (A), phenylalanine (F), serine (S), and threonine (T) and the mutation at codon 90 codes for a methionine (M).

70. The method of claim 68, wherein the **HIV**-infected patient is being treated with an antiretroviral agent.

71. A method for evaluating the biological effectiveness of a candidate **HIV protease** antiretroviral drug compound comprising: (a) introducing a resistance test vector comprising a patient-derived segment further comprising a mutation at codon 82 and additional mutations at one or more secondary positions and an indicator gene into a host cell; (b) culturing the host cell from step (a); (c) measuring the indicator in a target host cell; and (d) comparing the measurement of the indicator from step (c) with the measurement of the indicator measured when steps (a)-(c) are carried out in the absence of the candidate antiretroviral drug compound; wherein a test concentration of the candidate antiretroviral drug compound is present at steps (a)-(c); at steps (b)-(c); or at step (c).

72. A method for evaluating the biological effectiveness of a candidate **HIV protease** antiretroviral drug compound comprising: (a) introducing a resistance test vector comprising a patient-derived segment further comprising a mutation at codon 82 and secondary mutation(s) at codons 20, 24, 71, 54 and/or 10 or a combination thereof and an indicator gene into a host cell; (b) culturing the host cell from step (a); (c) measuring the indicator in a target host cell; and (d) comparing the measurement of the indicator from step (c) with the measurement of the indicator measured when steps (a)-(c) are carried out in the absence of the candidate antiretroviral drug compound; wherein a test concentration of the candidate antiretroviral drug compound is present at steps (a)-(c); at steps (b)-(c); or at step (c).

73. A method for evaluating the biological effectiveness of a candidate **HIV protease** antiretroviral drug compound comprising: (a) introducing a resistance test vector comprising a patient-derived segment further comprising a mutation at codon 90 and additional mutations at one or more secondary positions and an indicator gene into a host cell; (b) culturing the host cell from step (a); (c) measuring the indicator in a target host cell; and (d) comparing the measurement of the indicator from step (c) with the measurement of the indicator measured when steps (a)-(c) are carried; out in the absence of the candidate antiretroviral drug compound; wherein a test concentration of the candidate antiretroviral drug compound is present at steps (a)-(c); at steps (b)-(c); or at step (c).

74. A method for evaluating the biological effectiveness of a candidate **HIV protease** antiretroviral drug compound comprising: (a) introducing a resistance test vector comprising a patient-derived segment further comprising a mutation at codon 90 and secondary mutation(s) at codons 73, 71, 10 and/or 46 or a combination thereof and an indicator gene into a host cell; (b) culturing the host cell from step (a); (c) measuring the indicator in a target host cell; and (d)

comparing the measurement of the indicator from step (c) with the measurement of the indicator measured when steps (a)-(c) are carried out in the absence of the candidate antiretroviral drug compound; wherein a test concentration of the candidate antiretroviral drug compound is present at steps (a)-(c); at steps (b)-(c); or at step (c).

75. A method for evaluating the biological effectiveness of a candidate **HIV protease** antiretroviral drug compound comprising: (a) introducing a resistance test vector comprising a patient-derived segment further comprising a mutation at codons 82 and 90 and additional mutations at one or more secondary positions and an indicator gene into a host cell; (b) culturing the host cell from step (a); (c) measuring the indicator in a target host cell; and (d) comparing the measurement of the indicator from step (c) with the measurement of the indicator measured when steps (a)-(c) are carried out in the absence of the candidate antiretroviral drug compound; wherein a test concentration of the candidate antiretroviral drug compound is present at steps (a)-(c); at steps (b)-(c); or at step (c).

76. A resistance test vector comprising an **HIV** patient-derived segment further comprising **protease** having a mutation at codon 82 and an indicator gene, wherein the expression of the indicator gene is dependent upon the patient derived segment.

77. The resistance test vector of claim 76, wherein the patient-derived segment having a mutation at codon 82 further comprises at least one secondary mutation at a codon selected from the group consisting of 20, 24, 71, 54, 10 and a combination thereof.

78. The resistance test vector of claim 76, wherein the patient-derived segment having a mutation at codon 90 further comprises at least one secondary mutation at a codon selected from the group consisting of 73, 71, 46, 10 and a combination thereof.

79. A method for determining replication capacity for a patient's virus comprising: (a) introducing a resistance test vector comprising a patient derived segment and an indicator gene into a host cell; (b) culturing the host cell from (a); (c) harvesting viral particles from step (b) and infecting target host cells; (d) measuring expression of the indicator gene in the target host cell, wherein the expression of the indicator gene is dependent upon the patient-derived segment; (e) comparing the expression of the indicator gene from (d) with the expression of the indicator gene measured when steps (a) through (d) are carried out in a control resistance test vector; and (f) normalizing the expression of the indicator gene by measuring an amount of virus in step (c).

80. A method of assessing the effectiveness of **protease** antiretroviral therapy of an **HIV**-infected patient comprising: (a) collecting a biological sample from the **HIV**-infected patient; (b) evaluating whether the biological sample contains nucleic acid encoding **HIV protease** having a mutation at codon 82 or codon 90; and (c) determining changes in susceptibility to **protease** inhibitors.

81. The method of claim 80, wherein step (c) determines changes in susceptibility to saquinavir.

82. The method of claim 80, wherein the mutation at codon 82 codes for alanine (A), phenylalanine (F), serine (S), or threonine (T).

83. The method of claim 82, wherein the mutation at codon 82 is a substitution of alanine (A), phenylalanine (F), serine (S), or threonine (T) for valine(V).

84. The method of claim 80, wherein the mutation at codon 90 codes for methionine (M).

85. The method of claim 84, wherein the mutation at codon 90 is a substitution of methionine (M) for leucine (L)

86. A method for evaluating the biological effectiveness of a candidate **HIV protease** antiretroviral drug compound comprising: (a) introducing a resistance test vector comprising a patient-derived segment having nucleic acid encoding **HIV protease** with a mutation at codon 82 or codon 90 and an indicator gene into a host cell; (b) culturing the host cell from step (a); (c) measuring the indicator gene in a target host cell; and (d) comparing the measurement of the indicator gene from step (c) with the measurement of the indicator gene measured when steps (a)-(c) are carried out in the absence of the candidate antiretroviral drug compound; wherein a test concentration of the candidate antiretroviral drug compound is present at steps (a)-(c); at steps (b)-(c); or at step (c).

87. A resistance test vector comprising an **HIV** patient-derived segment further comprising **protease** having a mutation at codon 82 or codlon 90 and an indicator gene, wherein the expression of the indicator gene is dependent upon the patient-derived segment.

88. The resistance test vector of claim 87, wherein the patient-derived segment having a mutation at codon 82 codes for alanine (A), phenylalanine (F), serine (S), or threonine (T).

89. The resistance test vector of claim 88, wherein the patient-derived segment having a mutation at codon 82 is a substitution of alanine (A), phenylalanine (F), serine (S), or threonine (T) for valine(V).

90. The resistance test vector of claim 87, wherein the patient-derived segment having a mutation at codon 90 codes for methionine (M).

91. The resistance test vector of claim 90, wherein the patient-derived segment having a mutation at codon 90 is a substitution of methionine (M) for leucine (L).

92. A method for determining replication capacity for a patient's virus comprising: (a) introducing a resistance test vector comprising a patient-derived segment and an indicator gene into a host cell; (b) culturing the host cell from (a); (c) harvesting viral particles from step (b) and infecting target host cells; (d) measuring expression of the indicator gene in the target host cell, wherein the expression of the indicator gene is dependent upon the patient-derived segment; and (e) comparing the expression of the indicator gene from (d) with the expression of the indicator gene measured when steps (a) through (d) are carried out in a control resistance test vector.

93. The method of claim 92 further comprising the step of: (f) normalizing the expression of the indicator gene by measuring an amount of virus in step (c).

94. The method of claim 92 wherein the patient-derived segment comprises nucleic acid encoding **HIV** integrase having a mutation at codon 66.

95. The method of claim 92 wherein the patient-derived segment comprises nucleic acid encoding **HIV** integrase having a mutation at codon 154.

96. The method of claim 94 wherein the patient-derived segment comprises nucleic acid encoding **HIV** integrase having an additional mutation at codon 153.

97. The method of claim 94 wherein the patient-derived segment comprises nucleic acid encoding **HIV** integrase having an additional mutation at codon 154.

98. A method of assessing the effectiveness of **protease** antiretroviral therapy of an **HIV**-infected patient comprising: (a) collecting a biological sample from the **HIV**-infected patient; (b) evaluating whether the biological sample contains nucleic acid encoding **HIV protease** having a mutation at codon 82 and a secondary mutation at codons selected from the group consisting of 73, 55, 48, 20, 43, 53, 90, 13, 84, 23, 33, 74, 32, 39, 60, 36, and 35, or a mutation at codon 90 and a secondary mutation at codons selected from the group consisting of 53, 95, 54, 84, 82, 46, 13, 74, 55, 85, 20, 72, 62, 66, 84, 48, 33, 73, 71, 64, 93, 23, 58, and 36; and (c) determining a change in susceptibility to a **protease** inhibitor.

99. The method of claim 98, wherein the mutation at codon 82 is a substitution of alanine (A), phenylalanine (F), serine (S), or threonine (T) for valine(V) and the mutation at codon 90 is a substitution of methionine (M) for leucine (L)

100. The method of claim 99, wherein the **protease** inhibitor is selected from the group consisting of indinavir, amprenavir, and saquinavir.

101. The method of claim 100, having a mutation at codon 82 and a secondary mutation at codons selected from the group consisting of 84, 48, 23, 73, 53, 33, 74, 20, 90, 32 and 39 or a mutation at codon 90 and a secondary mutation at codons selected from the group consisting of 53, 66, 84, 54, 48, 33, 73, 20, 71, 64 and 93, wherein the **protease** inhibitor is saquinavir.

102. The method of claim 101, having a mutation at codon 82 and a secondary mutation at codons selected from the group consisting of 84, 48, 23, 73, 53, 33, 74, 20, and 90, or a mutation at codon 90 and a secondary mutation at codons selected from the group consisting of 53, 66, 84, 54, 48, 33, 73, 20, and 71, wherein the change in susceptibility in step (c) is a decrease in susceptibility to saquinavir.

103. The method of claim 101, having a mutation at codon 82 and a secondary mutation at codons 32 or 39, or a mutation at codon 90 and a secondary mutation at codons 64 or 93, wherein the change in susceptibility in step (c) is an increase in susceptibility to saquinavir.

104. The method of claim 100, having a mutation at codon 90 and a secondary mutation at codons selected from the group consisting of 53, 95, 54, 84, 82, 46, 13, and 74, wherein the **protease** inhibitor is indinavir.

105. The method of claim 104, having a mutation at codon 90 and a secondary mutation at codons selected from the group consisting of 53, 95, 54, 84, 82, and 46, wherein the change in susceptibility in step (c) is a decrease in susceptibility to indinavir.

106. The method of claim 104, having a mutation at codon 90 and a secondary mutation at codons 13 or 74, wherein the change in susceptibility in step (c) is an increase in susceptibility to indinavir.

107. The method of claim 100, having a mutation at codon 82 and a secondary mutation at codons selected from the group consisting of 73, 55, 48, 20, 43, 53, 90, 13, 48, 23, 84, 53, 74, 60, 33, 36, 35, 32, and 46 or a mutation at codon 90 and a secondary mutation at codons selected from the group consisting of 95, 55, 54, 82, 85, 84, 20, 72, 62, 74, 53, 48, 23, 58, 36, 64, 77, and 93.

108. The method of claim 107, wherein the **protease** inhibitor is selected from the group consisting of indinavir, amprenavir, and saquinavir.

109. The method of claim 108, wherein step (c) is determining a change in susceptibility to the **protease** inhibitor greater than 10 fold.

110. The method of claim 108, having a mutation at codon 82 and a secondary mutation at codons selected from the group consisting of 48, 23, 84, 53, 74, 20, 60, 33, 36, 35, or a mutation at codon 90 and a secondary mutation at codons selected from the group consisting of 84, 53, 48, 23, 58, 20, 36, and 54, wherein the change in susceptibility in step (c) is a decrease in susceptibility to saquinavir.

111. The method of claim 108, having a mutation at codon 82 and a secondary mutation at codons 32 or 46, or a mutation at codon 90 and a secondary mutation at codons 64, 77, or 93, wherein the change in susceptibility in step (c) is an increase in susceptibility to saquinavir.

112. The method of claim 108, having a mutation at codon 82 and a secondary mutation at codons selected from the group consisting of 73, 55, 48, 20, 43, 53, and 90, or a mutation at codon 90 and a secondary mutation at codons selected from the group consisting of 95, 55, 54, 82, 85, 84, 20, 72, and 62, wherein the change in susceptibility in step (c) is a decrease in susceptibility to indinavir.

113. The method of claim 108, having a mutation at codon 82 and a secondary mutation at codon 13, or a mutation at codon 90 and a secondary mutation at codon 74, wherein the change in susceptibility in step (c) is an increase in susceptibility to indinavir.

114. A method of assessing the effectiveness of **protease** antiretroviral therapy of an **HIV**-infected patient comprising: (a) collecting a biological sample from the **HIV**-infected patient; (b) evaluating whether the biological sample contains nucleic acid encoding **HIV protease** having a mutation at codon 90 and secondary mutations of at least three codons; and (c) determining a decrease in susceptibility to saqinavir.

115. The method of claim 114, wherein in the evaluating step (b), the nucleic acid encoding **HIV protease** has secondary mutations of at least five codons.

116. The method of claim 114, wherein the secondary mutation are selected from the group consisting of codons 10, 20, 52, 53, 54, 66, 71, 73 and 84.

117. A method of assessing the effectiveness of **protease** antiretroviral therapy of an **HIV**-infected patient comprising: (a) collecting a biological sample from the **HIV**-infected patient; (b) evaluating whether the biological sample contains nucleic acid encoding **HIV protease** having a mutation at codon 82 and secondary mutations at codons selected from the group consisting of 33, 23, 84, 32, 53, 90,

secondary mutations at codons selected from the group consisting of 89, 53, 84, 33, 92, 95, 54, 58, 46, 82, 36, 10, 62, 74, 15, 47, 66, 32, 55, 53, 13, and 69; and (c) determining a change in susceptibility to amprenavir.

118. The method of claim 117, wherein the mutation at codon 82 is a substitution of alanine (A), phenylalanine (F), serine (S), or threonine (T) for valine(V) and the mutation at codon 90 is a substitution of methionine (M) for leucine (L).

119. The method of claim 118, having a mutation at codon 82 and secondary mutations at codons selected from the group consisting of 33, 23, 84, 32, 53, 90, 37, 71, 46, 10, 54, 11, and 46, or a mutation at codon 90 and secondary mutations at codons selected from the group consisting of 89, 53, 84, 33, 92, 95, 54, 58, 46, 82, 36, 10, 62, 47, 66, 32, 55, 53, and 13; wherein the change in susceptibility in step (c) is a decrease in susceptibility to saquinavir.

120. The method of claim 118, having a mutation at codon 82 and a secondary mutation at codon 61, or a mutation at codon 90 and secondary mutations at codons 74, 15, or 69, wherein the change in susceptibility in step (c) is an increase in susceptibility to saquinavir.

121. A resistance test vector comprising an **HIV** patient-derived segment comprising nucleic acid encoding **protease** having a mutation at codon 82 and secondary mutations at codons selected from the group consisting of 73, 55, 48, 20, 43, 53, 90, 13, 84, 23, 33, 74, 32, 39, 60, 36, and 35, or a mutation at codon 90 and secondary mutations at codons selected from the group consisting of 53, 95, 54, 84, 82, 46, 13, 74, 55, 85, 20, 72, 62, 66, 84, 48, 33, 73, 71, 64, 93, 23, 58, and 36 and an indicator gene, wherein the expression of the indicator gene is dependent upon the patient-derived segment.

122. The resistance test vector of claim 121, wherein the mutation of the patient derived segment at codon 82 is a substitution of alanine (A), phenylalanine (F), serine (S), or threonine (T) for valine(V) and the mutation at codon 90 is a substitution of methionine (M) for leucine (L).

```
=> s (HIV?/clm or human immunodeficiency virus/clm)
          7950 HIV?/CLM
        109439 HUMAN/CLM
          3127 IMMUNODEFICIENCY/CLM
         19686 VIRUS/CLM
          2213 HUMAN IMMUNODEFICIENCY VIRUS/CLM
                 ((HUMAN(W)IMMUNODEFICIENCY(W)VIRUS)/CLM)
L5        8918 (HIV?/CLM OR HUMAN IMMUNODEFICIENCY VIRUS/CLM)

=> s l5 and (PR/clm or protease/clm)
          8975 PR/CLM
          8461 PROTEASE/CLM
L6        1383 L5 AND (PR/CLM OR PROTEASE/CLM)

=> s l6 and (hypersusceptib?/clm or increase? susceptibil?/clm)
             9 HYPERSUSCEPTIB?/CLM
        250620 INCREASE?/CLM
          3921 SUSCEPTIBIL?/CLM
           139 INCREASE? SUSCEPTIBIL?/CLM
                 ((INCREASE?(W)SUSCEPTIBIL?)/CLM)
L7           3 L6 AND (HYPERSUSCEPTIB?/CLM OR INCREASE? SUSCEPTIBIL?/CLM)

=> d his

     (FILE 'HOME' ENTERED AT 13:01:23 ON 23 JUL 2007)

     FILE 'USPATFULL' ENTERED AT 13:01:39 ON 23 JUL 2007
                E PARKIN NEIL T/IN
L1          27 S E3
L2          12 S L1 AND (PR/CLM OR PROTEASE/CLM)
L3          11 S L2 AND (HIV?/CLM OR HUMAN IMMUNODEFICIENCY VIRUS/CLM)
L4           3 S L3 AND (HYPERSUSCEPTIBIL?/CLM OR INCREASE? SUSCEPTIBILIT?/CLM
L5        8918 S (HIV?/CLM OR HUMAN IMMUNODEFICIENCY VIRUS/CLM)
L6        1383 S L5 AND (PR/CLM OR PROTEASE/CLM)
L7           3 S L6 AND (HYPERSUSCEPTIB?/CLM OR INCREASE? SUSCEPTIBIL?/CLM)

=> s l7 not l4
L8           0 L7 NOT L4

=> file wpids
COST IN U.S. DOLLARS                        SINCE FILE      TOTAL
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FULL ESTIMATED COST 20.70 20.91

FILE 'WPIDS' ENTERED AT 13:07:27 ON 23 JUL 2007
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FILE LAST UPDATED: 19 JUL 2007 <20070719/UP>
MOST RECENT THOMSON SCIENTIFIC UPDATE: 200746 <200746/DW>
DERWENT WORLD PATENTS INDEX SUBSCRIBER FILE, COVERS 1963 TO DATE

>>> IPC Reform backfile reclassification has been loaded to 31 May 2007. No update date (UP) has been created for the reclassified documents, but they can be identified by 20060101/UPIC and 20061231/UPIC and 20060601/UPIC. <<<

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>>> FOR DETAILS ON THE NEW AND ENHANCED DERWENT WORLD PATENTS INDEX PLEASE SEE
http://www.stn-international.de/stndatabases/details/dwpi_r.html <<<

```
=> e parkin n t/in
E1            1     PARKIN N F/IN
E2            4     PARKIN N J/IN
E3           27 --> PARKIN N T/IN
E4            1     PARKIN N T /IN
E5            3     PARKIN P/IN
E6            1     PARKIN P C/IN
E7            2     PARKIN P D/IN
E8           15     PARKIN R/IN
E9            1     PARKIN R C/IN
E10           4     PARKIN R E/IN
E11           3     PARKIN R G/IN
E12           2     PARKIN R L/IN

=> s e3
L9           27 "PARKIN N T"/IN

=> s l9 and (HIV or human immunodeficiency virus)
         24848 HIV
        213592 HUMAN
          8691 IMMUNODEFICIENCY
         50868 VIRUS
          5389 HUMAN IMMUNODEFICIENCY VIRUS
                 (HUMAN(W)IMMUNODEFICIENCY(W)VIRUS)
L10          17 L9 AND (HIV OR HUMAN IMMUNODEFICIENCY VIRUS)

=> s l10 and (PR or protease)
         16863 PR
         19359 PROTEASE
L11          12 L10 AND (PR OR PROTEASE)

=> s l11 and (hypersusceptib? or increas? susceptib?)
            16 HYPERSUSCEPTIB?
       1426776 INCREAS?
         28396 SUSCEPTIB?
           285 INCREAS? SUSCEPTIB?
                 (INCREAS?(W)SUSCEPTIB?)
L12           3 L11 AND (HYPERSUSCEPTIB? OR INCREAS? SUSCEPTIB?)

=> d l12,bib,ab,1-3

L12  ANSWER 1 OF 3  WPIDS COPYRIGHT 2007       THE THOMSON CORP on STN
Full Text
AN   2004-142896 [14]   WPIDS
DNC  C2004-057499 [14]
DNN  N2004-113938 [14]
TI   Determining if a HIV has increased likelihood of being
     hypersusceptible to treatment with a protease inhibitor comprises
     detecting if the protease encoded by the HIV exhibits the presence or
     absence of a mutation, which is not L33F
DC   B04; D16; S03
IN   CHAPPEY C; GAMARNIK A; GAMARNIK A V; GAMARNIK A V &amp;amp;; PARKIN N
     T; PAXINOS E; PETROPOULOS C J; WRIN M T
PA   (CHAP-I) CHAPPEY C; (GAMA-I) GAMARNIK A; (PARK-I) PARKIN N T; (PAXI-I)
     PAXINOS E; (PETR-I) PETROPOULOS C J; (VIRO-N) VIROLOGIC INC; (WRIN-I) WRIN
     M T
CYC  102
```

[illegible]
US 20040106106 A1 20040603 (200436) EN
AU 2003247790 A1 20040119 (200447) EN
EP 1552023 A2 20050713 (200546) EN
TW 2004011180 A 20040701 (200580) ZH
JP 2005536200 W 20051202 (200582) JA 39
CN 1688719 A 20051026 (200618) ZH
ADT WO 2004003512 A2 WO 2003-US21023 20030701; US 20040106106 A1 Provisional US 2002-393234P 20020701; AU 2003247790 A1 AU 2003-247790 20030701; EP 1552023 A2 EP 2003-762334 20030701; TW 2004011180 A TW 2003-117955 20030701; US 20040106106 A1 US 2003-612600 20030701; EP 1552023 A2 WO 2003-US21023 20030701; JP 2005536200 W WO 2003-US21023 20030701; JP 2005536200 W JP 2004-518236 20030701; CN 1688719 A CN 2003-820747 20030701
FDT AU 2003247790 A1 Based on WO 2004003512 A; EP 1552023 A2 Based on WO 2004003512 A; JP 2005536200 W Based on WO 2004003512 A
PRAI US 2002-393234P 20020701
US 2003-612600 20030701
AB WO 2004003512 A2 UPAB: 20060203
NOVELTY - Determining whether a **HIV** has an increased likelihood of being **hypersusceptible** to treatment with a **protease** inhibitor comprises detecting whether the **protease** encoded by the **HIV** exhibits the presence or absence of at least one mutation associated with **hypersusceptibility** to treatment with the **protease** inhibitor at amino acid position 16, 20, 33, 36, 37, 39, 45, 65, 69, 77, 89 or 93 of an amino acid sequence of the **protease**.
DETAILED DESCRIPTION - Determining whether a **HIV** has an increased likelihood of being **hypersusceptible** to treatment with a **protease** inhibitor comprises detecting whether the **protease** encoded by the **HIV** exhibits the presence or absence of at least one mutation associated with **hypersusceptibility** to treatment with the **protease** inhibitor at amino acid position 16, 20, 33, 36, 37, 39, 45, 65, 69, 77, 89 or 93 of an amino acid sequence of the **protease**, where the mutation indicates that the **HIV** has an increased likelihood of being **hypersusceptible** to treatment with the **protease** inhibitor, provided that the mutation is not L33F.
INDEPENDENT CLAIMS are also included for the following:
(1) a method for determining whether an individual infected with **HIV** has an increased likelihood of being **hypersusceptible** to treatment with a **protease** inhibitor;
(2) an isolated oligonucleotide between about 10-40 nucleotides long encoding a portion of an **HIV protease** that comprises the mutation;
(3) a method for determining whether an **HIV** has an increased likelihood of having a low level of reduced susceptibility to treatment with a **protease** inhibitor; and
(4) a method for determining whether an individual infected with **HIV** has an increased likelihood of having a low level of reduced susceptibility to treatment with a **protease** inhibitor.
ACTIVITY - Anti-**HIV**.
MECHANISM OF ACTION - **Protease** Inhibitor.
USE - Determining whether **HIV** has an increased likelihood of being **hypersusceptible** to treatment with a **protease** inhibitor (claimed).

L12 ANSWER 2 OF 3 WPIDS COPYRIGHT 2007 THE THOMSON CORP on STN
Full Text
AN 2003-239098 [23] WPIDS
CR 2002-362299; 2002-582333; 2001-071401
DNC C2003-061205 [23]
DNN N2003-190573 [23]
TI Assessing the effectiveness of **protease** antiretroviral therapy of an **HIV**-infected patient by collecting a plasma sample and evaluating whether the plasma sample contains nucleic acid encoding **HIV protease**
DC B04; D16; S03
IN **PARKIN N T**; ZEIRMANN R A; ZIERMANN R A
PA (PARK-I) PARKIN N T; (VIRO-N) VIROLOGIC INC; (ZIER-I) ZIERMANN R A
CYC 26
PIA WO 2002099387 A2 20021212 (200323)* EN 221[0]
US 20030108857 A1 20030612 (200340) EN
EP 1407042 A2 20040414 (200426) EN
US 20060035249 A1 20060216 (200614) EN
ADT WO 2002099387 A2 WO 2002-US18684 20020604; US 20030108857 A1 US 2001-874472 20010604; EP 1407042 A2 EP 2002-744311 20020604; EP 1407042 A2 WO 2002-US18684 20020604; US 20060035249 A1 Provisional US 1999-140483P 19990622; US 20060035249 A1 CIP of US 2000-591899 20000612; US 20060035249 A1 CIP of US 2000-663458 20000915; US 20060035249 A1 CIP of US 2001-766344 20010119; US 20060035249 A1 Cont of US 2001-874472 20010604; US 20060035249 A1 US 2005-140311 20050527
FDT EP 1407042 A2 Based on WO 2002099387 A; US 20060035249 A1 CIP of US 6869759 B
PRAI WO 2002-US1682 20020118
US 2001-874472 20010604
US 1999-140483P 19990622
US 2000-591899 20000612
US 2000-663458 20000915
US 2001-766344 20010119

AB WO 2002099387 A2 UPAB: 20060119
NOVELTY - Assessing (M1) effectiveness of **protease** antiretroviral therapy of an **HIV**-infected patient (I) comprises:
(a) collecting a biological (plasma) sample from (I);
(b) evaluating whether sample contains nucleic acid encoding **HIV protease** having a mutation at codons 88, 82 or 90; and
(c) determining **increased susceptibility** to amprenavir (for mutation at 88) or changed susceptibility to **protease** inhibitors.
DETAILED DESCRIPTION - INDEPENDENT CLAIMS are also included for the following:
(1) Evaluating (M2) the biological effectiveness of a candidate **HIV** antiretroviral drug;
(2) A resistance test vector comprising an **HIV**-patient-derived segment having a mutation at codon 88 and an indicator gene, where the expression of the indicator gene is dependent upon the patient derived segment;
(3) Evaluating (M3) the viral fitness of a patient's virus;
(4) Determining (M4) whether an HV virus obtained from a patient infected with **HIV** is resistant to IDV (indinavir), LPV, NPV or RTV; and
(5) Determining whether an **HIV** virus obtained from a patient infected with **HIV** is resistant to LPV and IND.
USE - The method is useful for assessing the effectiveness of **protease** antiretroviral therapy of an **HIV**-infected patient (claimed).

L12 ANSWER 3 OF 3 WPIDS COPYRIGHT 2007 THE THOMSON CORP on STN
Full Text
AN 2002-362299 [39] WPIDS
CR 2001-071401; 2002-582333; 2003-239098
DNC C2002-102536 [39]
TI Assessing effectiveness of **protease** antiretroviral therapy of **human immunodeficiency virus**-infected patient by evaluating whether patient has nucleic acid encoding **HIV protease** with mutation at codon 82, 90, 88, and secondary mutations
DC B04; D16
IN **PARKIN N T**; ZIERMANN R A
PA (VIRO-N) VIROLOGIC INC; (MONO-N) MONOGRAM BIOSCIENCES INC
CYC 96
PIA WO 2002022076 A2 20020321 (200239)* EN 220[6]
AU 2001090923 A 20020326 (200251) EN
EP 1322779 A2 20030702 (200344) EN
US 7186506 B1 20070306 (200718) EN
ADT WO 2002022076 A2 WO 2001-US28754 20010914; AU 2001090923 A AU 2001-90923 20010914; EP 1322779 A2 EP 2001-970982 20010914; EP 1322779 A2 WO 2001-US28754 20010914; US 7186506 B1 CIP of US 2000-591899 20000612; US 7186506 B1 CIP of WO 2000-US17178 20000622; US 7186506 B1 US 2000-663458 20000915
FDT AU 2001090923 A Based on WO 2002022076 A; EP 1322779 A2 Based on WO 2002022076 A; US 7186506 B1 CIP of US 6869759 B
PRAI US 2000-663458 20000915
US 2000-591899 20000612
WO 2000-US17178 20000622
AB WO 2002022076 A2 UPAB: 20060119
NOVELTY - Assessing effectiveness of **protease** (**PR**) antiretroviral therapy of **human immunodeficiency virus** (**HIV**)-infected patient (P) comprising evaluating whether a biological sample of (P) has a nucleic acid (I) encoding **HIV PR** having a mutation at codon 82/90, is new.
DETAILED DESCRIPTION - Assessing effectiveness of **protease** antiretroviral therapy of an **human immunodeficiency virus** (**HIV**)-infected patient comprising (M1-M2):
(a) collecting plasma sample from **HIV**-infected patient, evaluating whether plasma sample contains (I) having mutation at codon 88 and additional mutations at codons 63, 77, 46, 10, 20, and/or 36 or their combinations and determining **increased susceptibility** to amprenavir, and decreased susceptibility to nelfinavir and indinavir; or
(b) collecting a biological sample from the **HIV** infected patient, evaluating whether the biological sample contains (I) having mutation at codon 82 or 90 and determining changes in susceptibility to **PR** inhibitors, is new.
Optionally, (M2) involves collecting plasma sample from **HIV** infected patient and evaluating whether plasma sample contains (I) having:
(a) mutation at codon(s) 82 and/or 90 and secondary mutations and determining changes in susceptibility to ritonavir, nelfinavir, indinavir, saquinavir and amprenavir;
(b) mutation at codon 82 and additional mutation at codon 24 or at codon 71, or mutations at codons 54, 46, 10, and/or 63, and determining decreased susceptibility to indinavir;
(c) mutation at codon 82 and an additional mutation at codon 20 or at codon 36, mutations at codons 24, 71, 54, and/or 10 or their combinations, and determining decreased susceptibility to saquinavir;
(d) mutation at codon 82 or codon 90 and the number of additional mutations at secondary positions, and determining decreased susceptibility to indinavir and saquinavir;

(f) mutation at codon 90 and additional mutation at codon 73 or codon 71, or mutations at codons 77 and 10, and determining decreased susceptibility to saquinavir.

INDEPENDENT CLAIMS are also included for the following:

(1) resistance test vectors (II, III, IV) comprising **HIV** patient-derived segment further comprising **PR** having mutation at codon 88, codon 82, or codon 82 or codon 90 and an indicator gene, respectively, where the expression of the indicator gene is dependent upon the patient-derived segment;

(2) evaluating biological effectiveness of candidate **HIV PR** antiretroviral drug compound involves:

(a) introducing resistance test vector comprising patient-derived segment further comprising a mutation at:

(i) codon 90 and additional mutations at one or more secondary positions e.g., mutations at codons 73, 71, and/or 46 or their combination, and an indicator gene; or

(ii) at codons 82 and 90 and additional mutations at one or more secondary positions and an indicator gene, into a host cell;

(b) culturing the host cell;

(c) measuring the indicator in the target host cell; and

(d) comparing measurement of the indicator obtained from the above step with the measurement of the indicator when carrying out the above mentioned steps in the absence of candidate retroviral drug compound, where a test concentration of the candidate retroviral drug compound is present in all the three steps, or in two steps, or in one step before the comparison step; and

(3) determining (M3) replication capacity for a patient's virus involves:

(a) introducing a resistance test vector comprising a patient-derived segment and an indicator gene into a host cell;

(b) culturing the host cell;

(c) harvesting viral particles and infecting target host cells;

(d) measuring expression of the indicator gene which is dependent upon the patient-derived segment; and

(e) comparing the expression of the indicator gene measured in the above step with the expression of the indicator gene measured when all the above steps are carried out in a control resistance test vector.

USE - Assessing the effectiveness of **PR** antiretroviral therapy of an **HIV** infected patient who is being treated with antiretroviral agent. (II) is useful for evaluating the biological effectiveness of a candidate **HIV** antiretroviral drug compound which involves:

(1) introducing (II) comprising a patient-derived segment further comprising a mutation at codon 88 and mutation(s) at codons 63, 77, 46, 10, 20, and/or 36 or their combination, and an indicator gene into a host cell;

(2) culturing the host cell;

(3) measuring the indicator in the target host cell; and

(4) comparing measurement of the indicator obtained from the above step with the measurement of the indicator when carrying out the above mentioned steps in the absence of candidate retroviral drug compound, where a test concentration of the candidate retroviral drug compound is present at (1)-(3) at steps (2)-(3) or at step (3).

(II) is also useful for evaluating viral fitness of a patient's virus which involves introducing (II) and an indicator gene into a host cell, culturing host cell, measuring luciferase activity in target host cell in the absence of any antiretroviral drug, and comparing measurement of indicator from above step with measurement of indicator measured by carrying out all the above steps for a reference control in the absence of any retroviral drug and a reduction in the luciferase activity measured in presence of retroviral drug as compared to luciferase activity in absence of antiretroviral drug indicates a reduction in viral fitness. (III) is also useful for evaluating biological effectiveness of candidate **HIV PR** antiretroviral drug, as described above, where (III) employed in the method comprises patient-derived segment comprising mutation at codon 82 and secondary mutations at codons 20, 24, 71, 54, and/or 10 or their combination and an indicator gene (all claimed).

ADVANTAGE - The discovery of genetic changes in **HIV PR** that confers changes in susceptibility to antiretroviral therapy may be rapidly determined directly from patient plasma **HIV** RNA using phenotypic or genotypic methods.

=> d his

(FILE 'HOME' ENTERED AT 13:01:23 ON 23 JUL 2007)

FILE 'USPATFULL' ENTERED AT 13:01:39 ON 23 JUL 2007
E PARKIN NEIL T/IN
L1 27 S E3
L2 12 S L1 AND (PR/CLM OR PROTEASE/CLM)

```
L4            3 S L3 AND (HYPERSUSCEPTIBIL?/CLM OR INCREASE? SUSCEPTIBILIT?/CLM
L5         8918 S (HIV?/CLM OR HUMAN IMMUNODEFICIENCY VIRUS/CLM)
L6         1383 S L5 AND (PR/CLM OR PROTEASE/CLM)
L7            3 S L6 AND (HYPERSUSCEPTIB?/CLM OR INCREASE? SUSCEPTIBIL?/CLM)
L8            0 S L7 NOT L4

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                E PARKIN N T/IN
L9           27 S E3
L10          17 S L9 AND (HIV OR HUMAN IMMUNODEFICIENCY VIRUS)
L11          12 S L10 AND (PR OR PROTEASE)
L12           3 S L11 AND (HYPERSUSCEPTIB? OR INCREAS? SUSCEPTIB?)

=> s (HIV or human immunodeficiency virus)
         24848 HIV
        213592 HUMAN
          8691 IMMUNODEFICIENCY
         50868 VIRUS
          5389 HUMAN IMMUNODEFICIENCY VIRUS
                 (HUMAN(W)IMMUNODEFICIENCY(W)VIRUS)
L13      25650 (HIV OR HUMAN IMMUNODEFICIENCY VIRUS)

=> s l13 and (PR or protease)
         16863 PR
         19359 PROTEASE
L14       2534 L13 AND (PR OR PROTEASE)

=> s l14 and (hypersusceptib? or increase? susceptib?)
            16 HYPERSUSCEPTIB?
       1265491 INCREASE?
         28396 SUSCEPTIB?
           267 INCREASE? SUSCEPTIB?
                 (INCREASE?(W)SUSCEPTIB?)
L15           3 L14 AND (HYPERSUSCEPTIB? OR INCREASE? SUSCEPTIB?)

=> s l15 not l12
L16           0 L15 NOT L12

=> file medline
COST IN U.S. DOLLARS                          SINCE FILE      TOTAL
                                                 ENTRY    SESSION
FULL ESTIMATED COST                              30.96      51.87

FILE 'MEDLINE' ENTERED AT 13:09:37 ON 23 JUL 2007

 FILE LAST UPDATED: 21 Jul 2007 (20070721/UP).  FILE COVERS 1950 TO DATE.

  This file contains CAS Registry Numbers for easy and accurate
  substance identification.

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E1            9      PARKIN N/AU
E2            2      PARKIN N A/AU
E3           16 --> PARKIN N T/AU
E4           21      PARKIN NEIL/AU
E5           14      PARKIN NEIL T/AU
E6            9      PARKIN P/AU
E7            1      PARKIN P A/AU
E8           25      PARKIN P C/AU
E9           18      PARKIN P J/AU
E10           2      PARKIN PAMELA I/AU
E11           1      PARKIN PATRICIA/AU
E12          19      PARKIN PATRICIA C/AU

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            16 "PARKIN N T"/AU
            21 "PARKIN NEIL"/AU
            14 "PARKIN NEIL T"/AU
L17         51 ("PARKIN N T"/AU OR "PARKIN NEIL"/AU OR "PARKIN NEIL T"/AU)

=> s l17 and (PR or protease)
         22494 PR
         74806 PROTEASE
L18         27 L17 AND (PR OR PROTEASE)

=> s l18 and (PR/ab or protease/ab)
         19854 PR/AB
         54163 PROTEASE/AB
L19         22 L18 AND (PR/AB OR PROTEASE/AB)

=> s l19 and (HIV?/ab or human immunodeficiency virus/ab)
        113625 HIV?/AB
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         62585 IMMUNODEFICIENCY/AB
        279320 VIRUS/AB
         43781 HUMAN IMMUNODEFICIENCY VIRUS/AB
                 ((HUMAN(W)IMMUNODEFICIENCY(W)VIRUS)/AB)
L20         21 L19 AND (HIV?/AB OR HUMAN IMMUNODEFICIENCY VIRUS/AB)

=> s l20 and (hypersusceptib?/ab or increased susceptib?/ab)
           294 HYPERSUSCEPTIB?/AB
       1340060 INCREASED/AB
        159304 SUSCEPTIB?/AB
          6526 INCREASED SUSCEPTIB?/AB
                 ((INCREASED(W)SUSCEPTIB?)/AB)
L21          6 L20 AND (HYPERSUSCEPTIB?/AB OR INCREASED SUSCEPTIB?/AB)

=> d l21,cbib,ab,1-6

L21  ANSWER 1 OF 6     MEDLINE on STN
2007218983.     PubMed ID: 17314158.   Relative fitness and replication capacity
     of a multinucleoside analogue-resistant clinical human immunodeficiency
     virus type 1 isolate with a deletion of codon 69 in the reverse
     transcriptase coding region. Villena Cristina; Prado Julia G; Puertas
     Maria Carmen; Martinez Miguel Angel; Clotet Bonaventura; Ruiz Lidia;
     Parkin Neil T; Menendez-Arias Luis; Martinez-Picado Javier. (irsiCaixa
     Foundation, Hospital Germans Trias i Pujol, Ctra. de Canyet s/n, 08916
     Badalona, Spain. ) Journal of virology, (2007 May) Vol. 81, No. 9, pp.
     4713-21. Electronic Publication: 2007-02-21. Journal code: 0113724. ISSN:
     0022-538X. Pub. country: United States. Language: English.
AB   Deletions, insertions, and amino acid substitutions in the beta3-beta4
     hairpin loop-coding region of human immunodeficiency virus type 1
     (HIV-1) reverse transcriptase (RT) have been associated with resistance
     to nucleoside RT inhibitors when appearing in combination with other
     mutations in the RT-coding region. In this work, we have measured the in
     vivo fitness of HIV-1 variants containing a deletion of 3 nucleotides
     affecting codon 69 (Delta69) of the viral RT as well as the replication
     capacity (RC) ex vivo of a series of recombinant HIV-1 variants carrying
     an RT bearing the Delta69 deletion or the T69A mutation in a
     multidrug-resistant (MDR) sequence background, including the Q151M complex
     and substitutions M184V, K103N, Y181C, and G190A. Patient-derived viral
     clones having RTs with Delta69 together with S163I showed increased RCs
     under drug pressure. These data were consistent with the viral population
     dynamics observed in a long-term-treated HIV-1-infected patient. In the
     absence of drugs, viral clones containing T69A replicated more efficiently
     than those having Delta69, but only when patient-derived sequences
     corresponding to RT residues 248 to 527 were present. These effects could
     be attributed to a functional interaction between the C-terminal domain of
     the p66 subunit (RNase H domain) and the DNA polymerase domain of the RT.
     Finally, recombinant HIV-1 clones bearing RTs with MDR-associated
     mutations, including deletions at codon 69, showed increased
     susceptibilities to protease inhibitors in phenotypic assays. These
     effects correlated with impaired Gag cleavage and could be attributed to
     delayed maturation and decreased production of active protease in those
     variants.

L21  ANSWER 2 OF 6     MEDLINE on STN
2005393374.     PubMed ID: 16051856.   Evolution of human immunodeficiency virus
     type 1 protease genotypes and phenotypes in vivo under selective
     pressure of the protease inhibitor ritonavir. Resch Wolfgang; Parkin
     Neil; Watkins Terri; Harris Janera; Swanstrom Ronald. (Department of
     Biochemistry and Biophysics, University of North Carolina, Chapel Hill,
     NC, USA. ) Journal of virology, (2005 Aug) Vol. 79, No. 16, pp. 10638-49.
     Journal code: 0113724. ISSN: 0022-538X. Pub. country: United States.
     Language: English.
AB   We examined the population dynamics of human immunodeficiency virus
     type 1 pro variants during the evolution of resistance to the protease
     inhibitor ritonavir (RTV) in vivo. pro variants were followed in subjects
     who had added RTV to their previously failed reverse transcriptase
     inhibitor therapy using a heteroduplex tracking assay designed to detect
     common resistance-associated mutations. In most cases the initial variant
     appeared rapidly within 2 to 3 months followed by one or more subsequent
     population turnovers. Some of the subsequent transitions between variants
     were rapid, and some were prolonged with the coexistence of multiple
     variants. In several cases variants without resistance mutations
     persisted despite the emergence of new variants with an increasing number
     of resistance-associated mutations. Based on the rate of turnover of pro
     variants in the RTV-treated subjects we estimated that the mean fitness of
     newly emerging variants was increased 1.2-fold (range, 1.02 to 1.8)
     relative to their predecessors. A subset of pro genes was introduced into
     infectious molecular clones. The corresponding viruses displayed impaired
     replication capacity and reduced susceptibility to RTV. A subset of these
     clones also showed increased susceptibility to two nonnucleoside
     reverse transcriptase inhibitors and the protease inhibitor saquinavir.
     Finally, a significant correlation between the reduced replication
```

capacity and reduced processing at the gag-NC-p1 processing site was noted. Our results reveal a complexity of patterns in the evolution of resistance to a **protease** inhibitor. In addition, these results suggest that selection for resistance to one **protease** inhibitor can have pleiotropic effects that can affect fitness and susceptibility to other drugs.

L21 ANSWER 3 OF 6 MEDLINE on STN

2005223418. PubMed ID: 15857976. Phenotypic hypersusceptibility to multiple **protease** inhibitors and low replicative capacity in patients who are chronically infected with human immunodeficiency virus type 1. Martinez-Picado Javier; Wrin Terri; Frost Simon D W; Clotet Bonaventura; Ruiz Lidia; Brown Andrew J Leigh; Petropoulos Christos J; **Parkin Neil T.** (IrsiCaixa Foundation Hospital Germans Trias i Pujol, Ctra. de Canyet, s/n 08916 Badalona, Spain.. javiermp@ns.hugtip.scs.es) . Journal of virology, (2005 May) Vol. 79, No. 10, pp. 5907-13. Journal code: 0113724. ISSN: 0022-538X. Pub. country: United States. Language: English.

AB **Increased susceptibility** to the **protease** inhibitors saquinavir and amprenavir has been observed in **human immunodeficiency virus** type 1 (**HIV**-1) with specific mutations in **protease** (V82T and N88S). **Increased susceptibility** to ritonavir has also been described in some viruses from antiretroviral agent-naive patients with primary **HIV**-1 infection in association with combinations of amino acid changes at polymorphic sites in the **protease**. Many of the viruses displaying **increased susceptibility** to **protease** inhibitors also had low replication capacity. In this retrospective study, we analyze the drug susceptibility phenotype and the replication capacity of virus isolates obtained at the peaks of viremia during five consecutive structured treatment interruptions in 12 chronically **HIV**-1-infected patients. Ten out of 12 patients had at least one sample with **protease** inhibitor **hypersusceptibility** (change </=0.4-fold) to one or more **protease** inhibitor. **Hypersusceptibility** to different **protease** inhibitors was observed at variable frequency, ranging from 38% to amprenavir to 11% to nelfinavir. Pairwise comparisons between susceptibilities for the **protease** inhibitors showed a consistent correlation among all pairs. There was also a significant relationship between susceptibility to **protease** inhibitors and replication capacity in all patients. Replication capacity remained stable over the course of repetitive cycles of structured treatment interruptions. We could find no association between in vitro replication capacity and in vivo plasma viral load doubling time and CD4(+) and CD8(+) T-cell counts at each treatment interruption. Several mutations were associated with **hypersusceptibility** to each **protease** inhibitor in a univariate analysis. This study extends the association between **hypersusceptibility** to **protease** inhibitors and low replication capacity to virus isolated from chronically infected patients and highlights the complexity of determining the genetic basis of this phenomenon. The potential clinical relevance of **protease** inhibitor **hypersusceptibility** and low replication capacity to virologic response to **protease** inhibitor-based therapies deserves to be investigated further.

L21 ANSWER 4 OF 6 MEDLINE on STN

2004223112. PubMed ID: 15122516. Identification of I50L as the signature atazanavir (ATV)-resistance mutation in treatment-naive HIV-1-infected patients receiving ATV-containing regimens. Colonno Richard; Rose Ronald; McLaren Colin; Thiry Alexandra; **Parkin Neil**; Friborg Jacques. (Bristol-Myers Squibb Pharmaceutical Research Institute, Wallingford, Connecticut, USA.. richard.colonno@bms.com) . The Journal of infectious diseases, (2004 May 15) Vol. 189, No. 10, pp. 1802-10. Electronic Publication: 2004-04-27. Journal code: 0413675. ISSN: 0022-1899. Pub. country: United States. Language: English.

AB Atazanavir (ATV) is a once-daily **human immunodeficiency virus** (**HIV**) **protease** inhibitor (PI) shown to be effective and well tolerated. ATV has a distinct resistance profile relative to other PIs, with susceptibility maintained against 86% of isolates resistant to 1-2 PIs. Clinical isolates obtained from PI-naive patients designated as experiencing virologic failure while receiving ATV-containing regimens contained a unique isoleucine-to-leucine substitution at amino acid residue 50 (I50L) of the **HIV**-1 **protease**. The I50L substitution, observed in all isolates exhibiting phenotypic resistance to ATV, emerged in a variety of different backgrounds and was most frequently accompanied by A71V, K45R, and/or G73S. Viruses containing an I50L substitution were growth impaired, displayed ATV-specific resistance, and had **increased susceptibilities** (</=0.4 of reference strain) to other PIs. Comparison of viruses bearing I50L with those bearing I50V revealed specific resistance to ATV and amprenavir, respectively, with no evidence of cross-resistance. The unique I50L substitution is the signature mutation for resistance to ATV.

L21 ANSWER 5 OF 6 MEDLINE on STN

2002409408. PubMed ID: 12163585. Nelfinavir-resistant, amprenavir-hypersusceptible strains of human immunodeficiency virus type 1 carrying an N88S mutation in **protease** have reduced infectivity, reduced

replication capacity, and reduced fitness and process the Gag polyprotein precursor aberrantly. Resch Wolfgang; Ziermann Rainer; **Parkin Neil**; Gamarnik Andrea; Swanstrom Ronald. (Department of Biochemistry and Biophysics, University of North Carolina at Chapel Hill, Chapel Hill, North Carolina 27599, USA.) Journal of virology, (2002 Sep) Vol. 76, No. 17, pp. 8659-66. Journal code: 0113724. ISSN: 0022-538X. Pub. country: United States. Language: English.

AB The evolution of **human immunodeficiency virus** type 1 (**HIV**-1) strains with reduced susceptibility to **protease** inhibitors (PIs) is a major cause of PI treatment failure. A subset of subjects failing a therapy regimen containing the PI nelfinavir developed mutations at position 88 in the **protease** region. The N88S mutation occurring in some of these subjects induces amprenavir **hypersusceptibility** and a reduction of fitness and replication capacity. Here we demonstrate that substitutions L63P and V77I in **protease**, in combination, partially compensate for the loss of fitness, loss of replication capacity, loss of specific infectivity, and aberrant Gag processing induced by the N88S mutation. In addition, these mutations partially ablate amprenavir **hypersusceptibility**. Addition of mutation M46L to a strain harboring mutations L63P, V77I, and N88S resulted in a reduction of fitness and infectivity without changing Gag-processing efficiency, while amprenavir **hypersusceptibility** was further diminished. The ratio of reverse transcriptase activity to p24 protein was reduced in this strain compared to that in the other variants, suggesting that the M46L effect on fitness occurred through a mechanism different from a Gag-processing defect. We utilized these mutant strains to undertake a systematic comparison of indirect, single, cycle-based measures of fitness with direct, replication-based fitness assays and demonstrated that both yield consistent results. However, we observed that the magnitude of the fitness loss for one of the mutants varied depending on the assay used.

L21 ANSWER 6 OF 6 MEDLINE on STN

2002381110. PubMed ID: 12131189. Evolving patterns of HIV-1 resistance to antiretroviral agents in newly infected individuals. Simon Viviana; Vanderhoeven Jeroen; Hurley Arlene; Ramratnam Bharat; Louie Michael; Dawson Keith; **Parkin Neil**; Boden Daniel; Markowitz Martin. (Aaron Diamond AIDS Research Center, The Rockefeller University, New York 1016, USA.) AIDS (London, England), (2002 Jul 26) Vol. 16, No. 11, pp. 1511-9. Journal code: 8710219. ISSN: 0269-9370. Pub. country: England: United Kingdom. Language: English.

AB OBJECTIVE: To assess temporal changes in prevalence of transmitted **HIV**-1 drug resistance in a homogeneous cohort of newly infected individuals. METHODS: Pretreatment genotypic and phenotypic drug resistance was tested in 154 subjects with primary **HIV**-1 infection identified between 1995 and 2001 (group A; n = 76) and 1999 and 2001 (group B; n = 78). Sequence analysis was assessed by population-based sequencing. Virus susceptibility to antiretroviral agents was determined by the PhenoSense assay (ViroLogic). RESULTS: The frequency of resistance-associated mutations in **protease** (**PR**) and reverse transcriptase (RT) genes increased from 13.2% (1995-1998) to 19.7% (1999-2001). Although the overall prevalence of viruses with phenotypic resistance did not vary (1995-1998, 10.0%; 1999-2001, 10.8%), the distribution of drug classes changed [nucleoside RT inhibitor (NRTI): 8.3% to 2.7%; non-NRTI: 5.0% to 8.1%; **protease** inhibitors (PI): 1.7% to 5.4%]. The decrease of phenotypic resistance to NRTI in 1999-2001 was caused by the absence of transmitted lamivudine-resistant variants. Phenotypically susceptible variants with aspartic acid or serine residues at position 215 of RT (5.3%; P = 0.04) instead emerged. **Hypersusceptibility** to PI decreased from 18.3% to 5.4% (P = 0.02) while the amino acid substitutions in **PR** increased over time: M36I (6.6% to 19.7%) and A71V/T (3.9% to 15.8%). CONCLUSIONS: There was an increase in the number of **HIV**-1 variants with both genotypic and phenotypic resistance to non-NRTI and PI over time. Furthermore, viruses with altered genotypes compatible with thymidine analogue or PI exposure but susceptible phenotypes were seen in 1999-2001. The latter findings suggest transmission of viruses from subjects who have either changed or discontinued therapy.

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(FILE 'HOME' ENTERED AT 13:01:23 ON 23 JUL 2007)

FILE 'USPATFULL' ENTERED AT 13:01:39 ON 23 JUL 2007

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            E PARKIN NEIL T/IN
L1         27 S E3
L2         12 S L1 AND (PR/CLM OR PROTEASE/CLM)
L3         11 S L2 AND (HIV?/CLM OR HUMAN IMMUNODEFICIENCY VIRUS/CLM)
L4          3 S L3 AND (HYPERSUSCEPTIBIL?/CLM OR INCREASE? SUSCEPTIBILIT?/CLM
L5       8918 S (HIV?/CLM OR HUMAN IMMUNODEFICIENCY VIRUS/CLM)
L6       1383 S L5 AND (PR/CLM OR PROTEASE/CLM)
L7          3 S L6 AND (HYPERSUSCEPTIB?/CLM OR INCREASE? SUSCEPTIBIL?/CLM)
L8          0 S L7 NOT L4
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```
            E PARKIN N T/IN
L9          27 S E3
L10         17 S L9 AND (HIV OR HUMAN IMMUNODEFICIENCY VIRUS)
L11         12 S L10 AND (PR OR PROTEASE)
L12          3 S L11 AND (HYPERSUSCEPTIB? OR INCREAS? SUSCEPTIB?)
L13      25650 S (HIV OR HUMAN IMMUNODEFICIENCY VIRUS)
L14       2534 S L13 AND (PR OR PROTEASE)
L15          3 S L14 AND (HYPERSUSCEPTIB? OR INCREASE? SUSCEPTIB?)
L16          0 S L15 NOT L12

     FILE 'MEDLINE' ENTERED AT 13:09:37 ON 23 JUL 2007
            E PARKIN N T/AU
L17         51 S E3-E5
L18         27 S L17 AND (PR OR PROTEASE)
L19         22 S L18 AND (PR/AB OR PROTEASE/AB)
L20         21 S L19 AND (HIV?/AB OR HUMAN IMMUNODEFICIENCY VIRUS/AB)
L21          6 S L20 AND (HYPERSUSCEPTIB?/AB OR INCREASED SUSCEPTIB?/AB)

=> s (HIV?/ab or human immunodeficiency virus/ab)
       113625 HIV?/AB
       976871 HUMAN/AB
        62585 IMMUNODEFICIENCY/AB
       279320 VIRUS/AB
        43781 HUMAN IMMUNODEFICIENCY VIRUS/AB
              ((HUMAN(W)IMMUNODEFICIENCY(W)VIRUS)/AB)
L22    122819 (HIV?/AB OR HUMAN IMMUNODEFICIENCY VIRUS/AB)

=> s l22 and (PR/ab or protease/ab)
        19854 PR/AB
        54163 PROTEASE/AB
L23      7120 L22 AND (PR/AB OR PROTEASE/AB)

=> s l23 and (hypersuscepti?/ab or increased susceptibil?/ab)
          295 HYPERSUSCEPTI?/AB
      1340060 INCREASED/AB
        99840 SUSCEPTIBIL?/AB
         6523 INCREASED SUSCEPTIBIL?/AB
              ((INCREASED(W)SUSCEPTIBIL?)/AB)
L24         33 L23 AND (HYPERSUSCEPTI?/AB OR INCREASED SUSCEPTIBIL?/AB)

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L25         27 L24 NOT L21

=> d l25,cbib,ab,1-27

L25  ANSWER 1 OF 27     MEDLINE on STN
2007123971.     PubMed ID: 17280617.   Baseline resistance to nucleoside reverse
     transcriptase inhibitors fails to predict virologic response to
     combination therapy in children (PACTG 338). Fiscus Susan A; Kovacs
     Andrea; Petch Leslie A; Hu Chengcheng; Wiznia Andrew A; Mofenson Lynne M;
     Yogev Ram; McIntosh Kenneth; Pelton Stephen I; Napravnik Sonia; Stanley
     Kenneth; Nachman Sharon A. (Department of Microbiology and Immunology,
     University of North Carolina School of Medicine, Chapel Hill, NC, USA..
     susan_fiscus@med.unc.edu) . AIDS research and therapy, (2007) Vol. 4, pp.
     2.  Electronic Publication: 2007-02-06. Journal code: 101237921. E-ISSN:
     1742-6405. Pub. country: England: United Kingdom. Language: English.
AB   ABSTRACT: BACKGROUND: The association between baseline drug resistance
     mutations and subsequent increase in viral failure has not been
     established for HIV-infected children.  We evaluated drug resistance
     mutations at 39 codon sites (21 protease inhibitor (PI) resistant codons
     and 18 nucleoside reverse transcriptase inhibitor (NRTI) resistant codons)
     for 92 clinically stable NRTI-experienced, PI-naive HIV-infected
     children 2 to 17 years of age who were initiating new therapy with
     ritonavir plus zidovudine (ZDV) and lamivudine or plus stavudine.  The
     association between baseline drug resistance mutations and subsequent
     viral failure after 12 and 24 weeks of highly active antiretroviral
     therapy (HAART) was studied.  RESULTS: There were few primary PI
     associated mutations in this PI-naive population, but 84% had NRTI
     mutations - codons 215 (66%), 41 (42%), 67 (37%), 210 (33%) and 70 (32%).
     None of the specific baseline drug resistance mutations were associated
     with a higher rate of virologic failure after 12 or 24 weeks of HAART.
     Median week 12 viral load decreased as the total number of NRTI mutations
     at baseline increased (P = 0.006).  Specifically, a higher level of
     baseline ZDV resistance mutation was associated with a decrease in viral
     failure after 12 weeks on a ZDV-containing HAART regimen (P = 0.017).
     CONCLUSION: No increase was seen in the rate of viral failure after HAART
     associated with the presence of resistance mutations at baseline.  This
     paradoxical result may be due to adherence, replicative capacity, or ZDV
     hypersusceptibility to the new regimen.

L25  ANSWER 2 OF 27     MEDLINE on STN
2007023179.     PubMed ID: 17219733.   Novel drug resistance mutations in HIV:
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Valentina; Ceccherini-Silberstein Francesca. (Department of Experimental Medicine and Biochemical Sciences, University of Rome Tor Vergata, Italy.) AIDS reviews, (2006 Oct-Dec) Vol. 8, No. 4, pp. 179-90. Ref: 83. Journal code: 101134876. ISSN: 1139-6121. Pub. country: Spain. Language: English.

AB During its spread among humans, **HIV**-1 has developed an extraordinary degree of genetic diversity. The pol region encoding for viral enzymes such as the reverse transcriptase and the **protease,** and the env region encoding for the viral glycoprotein gp41 are subjected not only to natural variation, but also to the selection pressure imposed by the pharmacologic treatment. Under these conditions in **HIV**-1 infected people, the virus is able to escape from antiviral drugs by accumulating mutations, either alone or in clusters. The patterns of mutations accumulated by **HIV**-1 under drug pressure are quite variable, depending on the backbone of virus strains, the level and type of pharmacologic pressure, and the length of therapy. To date, a high number of mutations in **protease,** reverse transcriptase, and gp41 have been associated with reduced susceptibility to the antiretroviral drugs currently available. However, a number of studies continuously highlight the existence of additional mutations beyond those currently known to be involved in the development of drug resistance in vivo. Most of these so-called "novel" mutations are involved in agonistic correlations with the classical drug resistance mutations on divergent evolutionary pathways, and are associated with an increased resistance to specific drugs. At the same time, the presence of some novel mutations at therapeutic failure has also been significantly associated with an increase of viremia, thus suggesting that they may also play a compensatory role leading to improved viral replication. Interestingly, some natural polymorphisms in drug-naive patients have been significantly associated with the development of drug resistance mutations at failure, thus suggesting their ability to decrease the genetic barrier to the development of drug resistance. In contrast, other novel mutations are negatively associated with specific antiviral treatment, showing negative interactions with relevant drug resistance mutations, and are associated with **increased susceptibility** to specific drugs. This article reviews the importance of recognition and the clinical relevance of novel mutations involved in resistance to the currently used antiretroviral drugs, discussing in particular the role of novel drug resistance mutations in the reverse transcriptase enzyme. Such novel mutations should be considered for improved prediction of clinical response to antiretroviral drugs and for assessing the efficacy of next-generation drugs.

L25 ANSWER 3 OF 27 MEDLINE on STN

2006668239. PubMed ID: 17072129. Diminished selection for thymidine-analog mutations associated with the presence of M184V in Ethiopian children infected with HIV subtype C receiving lamivudine-containing therapy. Averbuch Diana; Schapiro Jonathan M; Lanier E Randall; Gradstein Serge; Gottesman Giora; Kedem Eynat; Einhorn Menachem; Grisaru-Soen Galia; Ofir Michal; Engelhard Dan; Grossman Zehava. (Hadassah University Medical Center, Jerusalem, Israel.) The Pediatric infectious disease journal, (2006 Nov) Vol. 25, No. 11, pp. 1049-56. Journal code: 8701858. ISSN: 0891-3668. Pub. country: United States. Language: English.

AB BACKGROUND: We retrospectively studied the effect of the lamivudine-induced reverse transcription mutation M184V on selection of thymidine analog mutations (TAMs) in **HIV** subtype C-infected children and on clinical outcome. METHODS: We genotyped 135 blood samples from 55 children. TAMs accumulation, viral load and clinical outcome were compared in children maintained on zidovudine/stavudine + lamivudine + **protease** inhibitor/nonnucleoside reverse transcriptase inhibitor (PI/NNRTI) despite loss of viral suppression and in children treated with, or switched to, other nucleoside reverse transcriptase inhibitors (NRTIs). Drug susceptibility and replication capacity of selected samples were measured. RESULTS: M184V developed in 18 of 22 of children who had received only zidovudine/stavudine + lamivudine + PI/NNRTI during a mean of 23.2 +/- 3.2 months versus in 3 of 14 children treated with other drugs and/or having multiple regimen changes (P = 0.001). TAMs appeared, respectively, in 2 of 22 versus 12 of 14 (P < 0.0001). The 2 groups did not differ significantly in baseline **HIV**-RNA or CD4 count, sampling time, and follow-up period. In M184V-containing samples, we found large reductions in susceptibility to lamivudine and emtricitabine but not to other NRTIs. When T215Y was present without M184V, susceptibility to zidovudine was reduced 8-fold. When both M184V + T215Y occurred, susceptibility to zidovudine was substantially increased. Average inhibition concentration 50 values were similar to those documented in the Stanford database for subtype B **HIV** with these mutation patterns. CONCLUSIONS: Maintaining a thymidine analog + lamivudine-based regimen reduced accumulation of TAMs and increased zidovudine susceptibility. This is likely the result of an **increased susceptibility** to thymidine analog (zidovudine) in the context of M184V documented here for the first time in subtype C-infected children. This retrospective study supports the strategy of maintaining lamivudine-containing therapy in subtype C-infected children. This strategy may be beneficially applied in the

regimen became available recently but further options are limited.

L25 ANSWER 4 OF 27 MEDLINE on STN
2006540005. PubMed ID: 16964826. Investigation of baseline susceptibility to protease inhibitors in HIV-1 subtypes C, F, G and CRF02_AG. Abecasis Ana B; Deforche Koen; Bacheler Lee T; McKenna Paula; Carvalho Ana Patricia; Gomes Perpetua; Vandamme Anne-Mieke; Camacho Ricardo Jorge. (Virology Laboratory, Hospital Egas Moniz, Lisbon, Portugal.. ana.abecasis@uz.kuleuven.ac.be) . Antiviral therapy, (2006) Vol. 11, No. 5, pp. 581-9. Journal code: 9815705. ISSN: 1359-6535. Pub. country: England: United Kingdom. Language: English.
AB OBJECTIVE: To compare baseline susceptibility to **protease** inhibitors among **HIV**-1 isolates of subtypes C, F, G and CRF02_AG, and to identify polymorphisms that determine the differences in susceptibility. METHODS: A total of 42 samples of drug-naive patients infected with subtypes G (n=19), CRF02_AG (n = 10), F (n = 6) and C (n = 7) were phenotyped and genotyped with the Antivirogram and the ViroSeq 2.0 genotyping system, respectively. A Bayesian network approach was used for a preliminary analysis of the collected data and the dependencies indicated by the network were statistically confirmed. RESULTS: CRF02_AG samples were found to be more susceptible to nelfinavir and ritonavir than other subtypes. **Hypersusceptibility** to these drugs was associated with the 70R polymorphism. 37D/S/T was associated with reduced susceptibility to indinavir and 89M with reduced susceptibility to lopinavir. Susceptibility to tipranavir was the lowest among the subtype F samples and the highest for subtype G samples, with samples carrying 57R being more susceptible than samples carrying 57K. CONCLUSIONS: Our study suggests that there are baseline susceptibility differences between subtypes and these differences are due to naturally occurring polymorphisms in these subtypes. The predictive value for phenotype of these polymorphisms was even valid in subtypes where these polymorphisms are less prevalent. Taking into account such polymorphisms should improve current algorithms for interpretation of genotyping results in a subtype-independent way.

L25 ANSWER 5 OF 27 MEDLINE on STN
2006148654. PubMed ID: 16464891. Susceptibility to protease inhibitors in HIV-2 primary isolates from patients failing antiretroviral therapy. Rodes Berta; Sheldon Julie; Toro Carlos; Jimenez Victoria; Alvarez Miguel Angel; Soriano Vincent. (Molecular Biology Laboratory, Department of Infectious Diseases, Hospital Carlos III, Madrid, Spain.. brodes.hciii@salud.madrid.org) . The Journal of antimicrobial chemotherapy, (2006 Apr) Vol. 57, No. 4, pp. 709-13. Electronic Publication: 2006-02-07. Journal code: 7513617. ISSN: 0305-7453. Pub. country: England: United Kingdom. Language: English.
AB BACKGROUND: Current **protease** inhibitors (PIs) are designed against **HIV**-1, and information on their performance against **HIV**-2 clinical isolates is scarce. METHODS: Genetic and phenotypic analyses using all available PIs were performed in five **HIV**-2 primary isolates from two patients on regular follow-up who failed PI-HAART. RESULTS: **HIV**-2 proteases before therapy showed amino acids associated with resistance in **HIV**-1 (pro10V, pro32I, pro36I, pro46I, pro47V, pro71V and pro73A). Phenotypic results showed that indinavir, saquinavir, lopinavir and tipranavir had full activity against wild-type **HIV**-2. However, a susceptibility reduction was noticed for nelfinavir (6.6-fold) and amprenavir (31-fold). During therapy with lopinavir, one patient developed proV47A, which translated into high-level resistance (13.4- to 41-fold) to indinavir, lopinavir and amprenavir, and **hypersusceptibility** to saquinavir. All isolates from the other patient had multiple mutations after several PIs failed (proV10I, proV33L, proI54M, proV71I and proI82F). The acquisition of mutations 54M and 82F along with naturally occurring changes resulted in multi-PI-resistant viruses (33- to >1000-fold), and only saquinavir retained full activity. CONCLUSIONS: Naturally occurring secondary mutations or polymorphisms in the **HIV**-2 **protease** may decrease the activity of nelfinavir and amprenavir. Moreover, upon selection of primary resistance mutations, pre-existing secondary changes might play an important role in the acquisition of a multi-PI resistance phenotype in **HIV**-2.

L25 ANSWER 6 OF 27 MEDLINE on STN
2005654091. PubMed ID: 16334989. Virological significance, prevalence and genetic basis of hypersusceptibility to nonnucleoside reverse transcriptase inhibitors. Tachedjian Gilda; Mijch Anne. (Molecular Interactions Group, Macfarlane Burnet Institute for Medical Research and Public Health, GPO Box 2284, Melbourne, Vic. 3001, Australia.. gildat@burnet.edu.au) . Sexual health, (2004) Vol. 1, No. 2, pp. 81-9. Ref: 68. Journal code: 101242667. ISSN: 1448-5028. Pub. country: Australia. Language: English.
AB Nonnucleoside reverse transcriptase inhibitors (NNRTI) are used to treat **HIV**-infected individuals in combination with nucleoside analogues (NRTI) and **protease** inhibitors. Long-term treatment with antiretroviral agents results in the emergence of strains with decreased susceptibility

(resistance) to the drugs and is one of the major factors in loss of drug efficacy. Conversely, there have been recent reports of **HIV** strains with **increased susceptibility** (**hypersusceptibility**) to NNRTIs. These isolates emerge in patients on long-term antiretroviral therapy particularly in individuals receiving NRTIs. The prevalence of NNRTI **hypersusceptibility** ranges between 17.5 and 50% in NRTI-treatment experienced compared to 10% in NRTI-naive patients. There is an inverse correlation between NNRTI **hypersusceptibility** and phenotypic NRTI resistance and a direct correlation between the number of NRTI resistance mutations present in the **HIV** reverse transcriptase. Re-sensitisation of phenotypic NNRTI resistance has been reported by NRTI mutations and is not likely to be detected using genotypic resistance assays. Recent studies demonstrate that NNRTI **hypersusceptible** virus at baseline is likely to predict better virological outcomes in patients on NNRTI-based salvage regimens compared to patients with NNRTI susceptible virus. These studies have implications for the sequence of antiretroviral drug use where patients may benefit from NRTI therapy before the introduction of NNRTIs, however more studies are needed to examine this treatment rationale.

L25 ANSWER 7 OF 27 MEDLINE on STN

2005642782. PubMed ID: 16327332. High rate of proV47A selection in HIV-2 patients failing lopinavir-based HAART. Rodes Berta; Toro Carlos; Sheldon Julie A; Jimenez Victoria; Mansinho Kamal; Soriano Vincent. (Molecular Biology Laboratory, Department of Infectious Diseases, Hospital Carlos III, Madrid, Spain.) AIDS (London, England), (2006 Jan 2) Vol. 20, No. 1, pp. 127-9. Journal code: 8710219. ISSN: 0269-9370. Pub. country: England: United Kingdom. Language: English.

AB We describe the emergence of the proV47A mutation in three out of five **HIV**-2-infected individuals failing lopinavir/ritonavir-based HAART. The appearance of such mutated variants resulted in high levels of phenotypic resistance to lopinavir, cross-resistance to indinavir, amprenavir, and **hypersusceptibility** to saquinavir. A search in **HIV**-2 databases revealed that proV47A is present in 8.6% of **protease** inhibitor (PI)-experienced patients but absent in all PI-naive patients. Its selection may be a common mutational pathway for developing resistance to lopinavir/ritonavir in **HIV**-2.

L25 ANSWER 8 OF 27 MEDLINE on STN

2005457809. PubMed ID: 16127059. Molecular basis for increased susceptibility of isolates with atazanavir resistance-conferring substitution I50L to other protease inhibitors. Yanchunas Joseph Jr; Langley David R; Tao Li; Rose Ronald E; Friborg Jacques; Colonno Richard J; Doyle Michael L. (Gene Expression and Protein Biochemistry Department, Bristol-Myers Squibb Pharmaceutical Research Institute, Princeton, NJ 08543-4000, USA.) Antimicrobial agents and chemotherapy, (2005 Sep) Vol. 49, No. 9, pp. 3825-32. Journal code: 0315061. ISSN: 0066-4804. Pub. country: United States. Language: English.

AB **Protease** inhibitors (PIs) are highly effective drugs against the **human immunodeficiency virus** (**HIV**), yet long-term therapeutic use is limited by emergence of **HIV** type 1 (**HIV**-1) **protease** substitutions that confer cross-resistance to multiple **protease** inhibitor drugs. Atazanavir is a highly potent **HIV protease** inhibitor with a distinct resistance profile that includes effectiveness against most **HIV**-1 isolates resistant to one or two PIs. The signature resistance substitution for atazanavir is I50L, and it is frequently (53%) accompanied by a compensatory A71V substitution that helps restore viability and increases atazanavir resistance levels. We measured the binding affinities of wild-type (WT) and I50L/A71V **HIV**-1 proteases to atazanavir and other currently approved PIs (ritonavir, lopinavir, saquinavir, nelfinavir, indinavir, and amprenavir) by isothermal titration calorimetry. Remarkably, we find that all of the PIs have 2- to 10-fold increased affinities for I50L/A71V **protease**, except for atazanavir. The results are also manifested by thermal stability measures of affinity for WT and I50L/A71V proteases. Additional biophysical and enzyme kinetics experiments show I50L/A71V **protease** is a stable enzyme with catalytic activity that is slightly reduced (34%) relative to the WT. Computational modeling reveals that the unique resistance phenotype of I50L/A71V **protease** likely originates from bulky tert-butyl groups at P2 and P2' (specific to atazanavir) that sterically clash with methyl groups on residue L50. The results of this study provide a molecular understanding of the novel **hypersusceptibility** of atazanavir-resistant I50L/A71V-containing clinical isolates to other currently approved PIs.

L25 ANSWER 9 OF 27 MEDLINE on STN

2005457808. PubMed ID: 16127058. Atazanavir signature I50L resistance substitution accounts for unique phenotype of increased susceptibility to other protease inhibitors in a variety of human immunodeficiency virus type 1 genetic backbones. Weinheimer S; Discotto L; Friborg J; Yang H; Colonno R. (Bristol-Myers Squibb Pharmaceutical Research Institute, Wallingford, Conn, USA.) Antimicrobial agents and chemotherapy, (2005 Sep) Vol. 49, No. 9, pp. 3816-24. Journal code: 0315061. ISSN: 0066-4804. Pub. country: United States. Language: English.

AB Substitution of leucine for isoleucine at residue 50 (I50L) of **human**

substitution for atazanavir (ATV) resistance. A unique phenotypic profile has been associated with viruses containing the I50L substitution, which produces ATV-specific resistance and **increased susceptibility** to most other approved **HIV protease** inhibitors (PIs). The basis for this unique phenotype has not been clearly elucidated. In this report, a direct effect of I50L on the susceptibility to the PI class is described. Cell-based **protease** assays using wild-type and PI-resistant proteases from laboratory and clinical isolates and in vitro antiviral assays were used to demonstrate a strong concordance between changes in PI susceptibility at the level of **protease** inhibition and changes in susceptibility observed at the level of virus infection. The results show that the induction of ATV resistance and **increased susceptibility** to other PIs by the I50L substitution is likely determined at the level of **protease** inhibition. Moreover, the I50L substitution functions to increase PI susceptibility even in the presence of other primary and secondary PI resistance substitutions. These findings may have implications regarding the optimal sequencing of PI therapies necessary to preserve PI treatment options of patients with ATV-resistant **HIV** infections.

L25 ANSWER 10 OF 27 MEDLINE on STN

2005337401. PubMed ID: 15937277. Structural analysis of an HIV-1 protease I47A mutant resistant to the protease inhibitor lopinavir. Kagan Ron M; Shenderovich Mark D; Heseltine Peter N R; Ramnarayan Kal. (Department of Infectious Diseases, Quest Diagnostics Inc., San Juan Capistrano, CA 92675, USA.. kaganr@questdiagnostics.com) . Protein science : a publication of the Protein Society, (2005 Jul) Vol. 14, No. 7, pp. 1870-8. Electronic Publication: 2005-06-03. Journal code: 9211750. ISSN: 0961-8368. Pub. country: United States. Language: English.

AB We have identified a rare **HIV-1 protease** (**PR**) mutation, I47A, associated with a high level of resistance to the **protease** inhibitor lopinavir (LPV) and with **hypersusceptibility** to the **protease** inhibitor saquinavir (SQV). The I47A mutation was found in 99 of 112,198 clinical specimens genotyped after LPV became available in late 2000, but in none of 24,426 clinical samples genotyped from 1998 to October 2000. Phenotypic data obtained for five I47A mutants showed unexpected resistance to LPV (86- to >110-fold) and **hypersusceptibility** to SQV (0.1- to 0.7-fold). Molecular modeling and energy calculations for these mutants using our structural phenotyping methodology showed an increase in the binding energy of LPV by 1.9-3.1 kcal/mol with respect to the wild type complex, corresponding to a 20- to >100-fold decrease in binding affinity, consistent with the observed high levels of LPV resistance. In the WT **PR**-LPV complex, the Ile 47 side chain is positioned close to the phenoxyacetyl moiety of LPV and its van der Waals interactions contribute significantly to the ligand binding. These interactions are lost for the smaller Ala 47 residue. Calculated binding energy changes for SQV ranged from -0.4 to -1.2 kcal/mol. In the mutant I47A **PR**-SQV complexes, the **PR** flaps are packed more tightly around SQV than in the WT complex, resulting in the formation of additional hydrogen bonds that increase binding affinity of SQV consistent with phenotypic **hypersusceptibility**. The emergence of mutations at **PR** residue 47 strongly correlates with increasing prescriptions of LPV (Spearman correlation r(s) = 0.96, P < .0001).

L25 ANSWER 11 OF 27 MEDLINE on STN

2005250270. PubMed ID: 15889533. HIV-1 subtype C drug-resistance background among ARV-naive adults in Botswana. Bussmann Hermann; Novitsky Vladimir; Wester William; Peter Trevor; Masupu Kereng; Gabaitiri Lesego; Kim Soyeon; Gaseitsiwe Simane; Ndungu Thumbi; Marlink Richard; Thior Ibou; Essex Max. (Botswana-Harvard School of Public Health AIDS Initiative Partnership, Gaborone, Botswana.) Antiviral chemistry & chemotherapy, (2005) Vol. 16, No. 2, pp. 103-15. Journal code: 9009212. ISSN: 0956-3202. Pub. country: England: United Kingdom. Language: English.

AB Current **HIV-1** antiretroviral (ARV) drug resistance knowledge is limited to **HIV-1** subtype B (**HIV-1B**). We addressed whether unique genetic and phenotypic properties of **HIV-1** subtype C (**HIV-1C**), southern Africa's most prevalent subtype, may foment earlier and/or distinct resistance mutations. Population-level **HIV-1C** genotypes were evaluated with respect to drug resistance prevalence before Botswana's public ARV treatment programme began. Viruses were genotyped from 11 representative districts of northern and southern Botswana, and consensus sequences from these 71 individuals and 51 previously reported sequences from **HIV**-positive blood donors were constructed. Phylogenetic analysis classified all 71 sequences but one, which exhibited pol gene mosaicism, as **HIV-1C**. The **protease** and reverse transcriptase coding region had no detectable known primary mutations associated with **HIV-1B protease** inhibitor (PI) drug resistance. Secondary mutations associated with PI drug resistance were found in all sequences. Several **HIV-1C**-specific polymorphic sites were found across the pol gene. Northern and southern Botswana viral sequences showed no significant differences from each other. Population genotyping shows that, without countrywide ARV treatment, **HIV-1C**-infected Batswana harbour virtually no primary

mutations known to confer resistance to the three major HIV-1B RT drug classes. Some secondary PI mutations and polymorphic sites in the **protease** enzyme necessitate continuous population monitoring, particularly after introduction of countrywide ARV treatment in Botswana. Although its PI resistance development rate and kinetics are not known, our data may suggest **increased susceptibility** and readiness of **HIV**-1C to develop resistance under drug pressure when the PI class of drugs is used.

L25 ANSWER 12 OF 27 MEDLINE on STN
2004632442. PubMed ID: 15609242. Influence of indinavir on virulence and growth of Cryptococcus neoformans. Monari Claudia; Pericolini Eva; Bistoni Giovanni; Cenci Elio; Bistoni Francesco; Vecchiarelli Anna. (Microbiology Section, Department of Experimental Medicine and Biochemical Sciences, University of Perugia, Via del Giochetto, 06122 Perugia, Italy.) The Journal of infectious diseases, (2005 Jan 15) Vol. 191, No. 2, pp. 307-11. Electronic Publication: 2004-12-09. Journal code: 0413675. ISSN: 0022-1899. Pub. country: United States. Language: English.
AB Indinavir selectively inhibited production of some virulence factors of Cryptococcus neoformans, such as urease and **protease**, but not melanin and phospholipase; moreover, it interfered with capsule formation. These effects led to **increased susceptibility** of C. neoformans to intracellular killing by natural effector cells. Prolonged incubation with indinavir resulted in inhibition of fungal growth. Indinavir can attenuate the virulence of the fungus, thus augmenting its susceptibility to the antimicrobial activity of natural effector cells. The reduction in cryptococcal infections in **human immunodeficiency virus**-positive patients might also be related to the antifungal activity of highly active antiretroviral therapy.

L25 ANSWER 13 OF 27 MEDLINE on STN
2004512647. PubMed ID: 15482137. Atazanavir: improving the HIV protease inhibitor class. Becker Stephen. (University of California, San Francisco and Pacific Horizon Medical Group, Inc., San Francisco, CA, USA.. slbecker@mindspring.com) . Expert review of anti-infective therapy, (2003 Oct) Vol. 1, No. 3, pp. 403-13. Ref: 45. Journal code: 101181284. ISSN: 1478-7210. Pub. country: England: United Kingdom. Language: English.
AB **Protease** inhibitors are potent agents against **HIV** but their use is constrained by poor pharmacokinetics, cross-resistance and metabolic toxicities. Atazanavir [Reyataz] is a new **protease** inhibitors with once-daily dosing and minimal lipid and glycemic effects. Resistance studies of clinical isolates reveal a mutational pattern distinctive from that of other **protease** inhibitors. Atazanavir selects for the I50L mutation in **HIV protease** that confers **increased susceptibility** to other **protease** inhibitors in vitro. Clinical trials have shown comparable efficacy to nelfinavir (Viracept) and efavirenz (Sustiva) in treatment-naive patients, and in preliminary studies, ritonavir-boosted atazanavir is effective in patients failing previous **protease** inhibitor-containing regimens. Reversible elevations in bilirubin occur in some patients but are not associated with hepatic injury. Atazanavir improves upon aspects of currently-available **protease** inhibitors and appears useful for initial and possibly subsequent **HIV** therapy.

L25 ANSWER 14 OF 27 MEDLINE on STN
2004089533. PubMed ID: 14963120. Genetic basis of hypersusceptibility to protease inhibitors and low replicative capacity of human immunodeficiency virus type 1 strains in primary infection. Leigh Brown Andrew J; Frost Simon D W; Good Benjamin; Daar Eric S; Simon Viviana; Markowitz Martin; Collier Ann C; Connick Elizabeth; Conway Brian; Margolick Joseph B; Routy Jean-Pierre; Corbeil Jacques; Hellmann Nicholas S; Richman Douglas D; Little Susan J. (University of Edinburgh, Edinburgh, Scotland.. A.Leigh-Brown@ed.ac.uk) . Journal of virology, (2004 Mar) Vol. 78, No. 5, pp. 2242-6. Journal code: 0113724. ISSN: 0022-538X. Pub. country: United States. Language: English.
AB The initial virus strains from as many as 12% of individuals with primary **human immunodeficiency virus** (**HIV**) infection have a 50% inhibitory concentration </=0.4-fold that of **HIV** type 1(NL4-3) (**HIV**-1(NL4-3)) to ritonavir (**hypersusceptibility** [HS]). There is also substantial variation in replicative capacity (RC) or an in vitro assay of the contributions of **protease** (**PR**) and reverse transcriptase to viral fitness. In chronically infected antiretrovirally treated patients, amprenavir HS has been associated with the mutation N88S in **PR**, but this mutation is not seen in untreated patients. In this study, virus strains from 182 cases of primary **HIV** infection were analyzed, and a highly significant association between HS and low RC (</=10% that of **HIV**-1(NL4-3)) was observed (P < 10(-6)). Multivariate analysis was used to determine the genotypic basis of ritonavir HS, analyzing all polymorphic amino acid sites and insertions from p7gag through **PR**. Decision tree models developed on the entire Gag-plus-**PR** data set and on **PR** alone gave overall correct classifications of 73 and 72%, respectively, on cross-validation. They were also able to predict low RC, with sensitivities of 69 and 62% and specificities of 84 and 70%, respectively. The analysis shows that ritonavir HS in untreated primary

combinations of amino acids at polymorphic sites and that the same genotypes which confer HS to **PR** inhibitors confer low RC. This supports the view that variation in **PR** function is directly responsible for variation in fitness among strains in primary infection.

L25 ANSWER 15 OF 27 MEDLINE on STN
2003537074. PubMed ID: 14616723. New patterns of HIV-1 resistance during HAART. Fumero E; Podzamczer D. (Infectious Disease Service, Hospital Universitari de Bellvitge, Barcelona, Spain.) Clinical microbiology and infection : the official publication of the European Society of Clinical Microbiology and Infectious Diseases, (2003 Nov) Vol. 9, No. 11, pp. 1077-84. Ref: 59. Journal code: 9516420. ISSN: 1198-743X. Pub. country: France. Language: English.

AB **HIV**-1 resistance and subsequent virologic failure occur in a substantial proportion of **HIV**-infected patients receiving HAART regimens. In the present article, we summarize new data on resistance to current and forthcoming antiretroviral drugs which will help in the interpretation of the results of resistance tests and the individualization of therapy. Nucleoside analog mutations (NAMs) (M41L, D67N, K70R, L210W, T215Y/F and K219Q/E) are associated with reduced susceptibility to most nucleoside analogs and the nucleotide tenofovir. This recently approved drug has shown a reduced virologic response in the presence of three or more NAMs, including M41L or L210W, as well as in the presence of T69 insertions. **Hypersusceptibility** (IC50 < 0.5) to non-nucleoside reverse transcriptase inhibitors (NNRTIs) has recently been described in association with increased resistance to nucleoside analogs, and it seems to enhance the immunologic and virologic reponses in patients receiving efavirenz-containing regimens. New **protease** inhibitors (PIs) have a lower cross-resistance profile, although more clinical data are needed to establish appropriate PI sequencing to promote sustained virologic success. Cross-resistance between amprenavir (APV) and lopinavir (LPV/r) in the presence of only four APV-related mutations has been described, suggesting that phenotypic tests should be applied before prescribing LPV/r to APV-experienced patients. Resistance to the new entry inhibitor class compound T-20 (enfuvirtide) has also been detected.

L25 ANSWER 16 OF 27 MEDLINE on STN
2003409604. PubMed ID: 12936979. In vitro hypersusceptibility of human immunodeficiency virus type 1 subtype C protease to lopinavir. Gonzalez Luis M F; Brindeiro Rodrigo M; Tarin Michelle; Calazans Alexandre; Soares Marcelo A; Cassol Sharon; Tanuri Amilcar. (Departamento de Genetica, Universidade Federal do Rio de Janeiro, CCS, Bloco A, Cidade Universitaria, Ilha do Fundao, 21944-970 Rio de Janeiro, RJ, Brazil.) Antimicrobial agents and chemotherapy, (2003 Sep) Vol. 47, No. 9, pp. 2817-22. Journal code: 0315061. ISSN: 0066-4804. Pub. country: United States. Language: English.

AB In order to characterize the impact of genetic polymorphisms on the susceptibility of subtype C strains of **human immunodeficiency virus** type 1 to **protease** inhibitors (PIs), a subtype B **protease** that originated from an infectious clone was modified through site-directed mutagenesis to include the amino acid residue signatures of subtype C viruses (I15V, M36I, R41K, H69K, L89 M) with (clone C6) or without (clone C5) an I93L polymorphism present as a molecular signature of the worldwide subtype C **protease**. Their susceptibilities to commercially available PIs were measured by a recombinant virus phenotyping assay. We could not detect any differences in the 50% inhibitory concentration (IC(50)s) of amprenavir, indinavir, ritonavir, saquinavir, and nelfinavir for the clones analyzed. However, we did observe **hypersusceptibility** to lopinavir solely in clone C6, which includes the I93L substitution (a 2.6-fold decrease in the IC(50) compared to that for the subtype B reference strain). The same phenotypic behavior was observed for 11 Brazilian and South African clinical isolates tested, in which only subtype C isolates carrying the I93L mutation presented significant **hypersusceptibility** to lopinavir.

L25 ANSWER 17 OF 27 MEDLINE on STN
2002739617. PubMed ID: 12502865. Amino acid substitutions at position 190 of human immunodeficiency virus type 1 reverse transcriptase increase susceptibility to delavirdine and impair virus replication. Huang Wei; Gamarnik Andrea; Limoli Kay; Petropoulos Christos J; Whitcomb Jeannette M. (Department of Research and Development, ViroLogic, Inc., South San Francisco, California 94080, USA.) Journal of virology, (2003 Jan) Vol. 77, No. 2, pp. 1512-23. Journal code: 0113724. ISSN: 0022-538X. Pub. country: United States. Language: English.

AB Suboptimal treatment of **human immunodeficiency virus** type 1 (**HIV**-1) infection with nonnucleoside reverse transcriptase inhibitors (NNRTI) often results in the rapid selection of drug-resistant virus. Several amino acid substitutions at position 190 of reverse transcriptase (RT) have been associated with reduced susceptibility to the NNRTI, especially nevirapine (NVP) and efavirenz (EFV). In the present study, the effects of various 190 substitutions observed in viruses obtained from NNRTI-experienced patients were characterized with patient-derived **HIV**

isolates and confirmed with a panel of isogenic viruses. Compared to wild-type **HIV**, which has a glycine at position 190 (G190), viruses with 190 substitutions (A, C, Q, S, V, E, or T, collectively referred to as G190X substitutions) were markedly less susceptible to NVP and EFV. In contrast, delavirdine (DLV) susceptibility of these G190X viruses increased from 3 to 300-fold (**hypersusceptible**) or was only slightly decreased. The replication capacity of viruses with certain 190 substitutions (C, Q, V, T, and E) was severely impaired and was correlated with reduced virion-associated RT activity and incomplete **protease** (**PR**) processing of the viral p55(gag) polyprotein. These defects were the result of inadequate p160(gagpol) incorporation into virions. Compensatory mutations within RT and **PR** improved replication capacity, p55(gag) processing, and RT activity, presumably through increased incorporation of p160(gagpol) into virions. We observe an inverse relationship between the degree of NVP and EFV resistance and the impairment of viral replication in viruses with substitutions at 190 in RT. These observations may have important implications for the future design and development of antiretroviral drugs that restrict the outgrowth of resistant variants with high replication capacity.

L25 ANSWER 18 OF 27 MEDLINE on STN

2002352909. PubMed ID: 12095381. Dual vs single protease inhibitor therapy following antiretroviral treatment failure: a randomized trial. Hammer Scott M; Vaida Florin; Bennett Kara K; Holohan Mary K; Sheiner Lewis; Eron Joseph J; Wheat Lawrence Joseph; Mitsuyasu Ronald T; Gulick Roy M; Valentine Fred T; Aberg Judith A; Rogers Michael D; Karol Cheryl N; Saah Alfred J; Lewis Ronald H; Bessen Laura J; Brosgart Carol; DeGruttola Victor; Mellors John W. (Division of Infectious Diseases, Department of Medicien, Columbia University College of Physicians and Surgeons, 630 W 168th St, New York, NY 10032, USA. (AIDS Clinical Trials Group 398 Study Team). smh48@columbia.edu) . JAMA : the journal of the American Medical Association, (2002 Jul 10) Vol. 288, No. 2, pp. 169-80. Journal code: 7501160. ISSN: 0098-7484. Pub. country: United States. Language: English.

AB CONTEXT: Management of antiretroviral treatment failure in patients receiving **protease** inhibitor (PI)-containing regimens is a therapeutic challenge. OBJECTIVE: To assess whether adding a second PI improves antiviral efficacy of a 4-drug combination in patients with virologic failure while taking a PI-containing regimen. DESIGN: Multicenter, randomized, 4-arm trial, double-blind and placebo-controlled for second PI, conducted between October 1998 and April 2000, for which there was a 24-week primary analysis with extension to 48 weeks. SETTING: Thirty-one participating AIDS (acquired immunodeficiency syndrome) Clinical Trials Units in the United States. PARTICIPANTS: A total of 481 **human immunodeficiency virus** (**HIV**)-infected persons with prior exposure to a maximum of 3 PIs and viral load above 1000 copies/mL. INTERVENTION: Selectively randomized assignment (per prior PI exposure) to saquinavir (n = 116); indinavir (n = 69); nelfinavir (n = 139); or placebo twice per day (n = 157); in combination with amprenavir, abacavir, efavirenz, and adefovir dipivoxil. MAIN OUTCOME MEASURES: Primary efficacy analysis involved the proportion with viral load below 200 copies/mL at 24 weeks. Other measures were changes in viral load and CD4 cell count from baseline, adverse events, and **HIV** drug susceptibility. RESULTS: Of 481 patients, 148 (31%) had a viral load below 200 copies/mL at week 24. The proportions of patients with a viral load below 200 copies/mL in the saquinavir, indinavir, nelfinavir, and placebo arms were 34% (40/116), 36% (25/69), 34% (47/139), and 23% (36/157), respectively. The proportion in the combined dual-PI arms was higher than in the amprenavir-plus-placebo arm (35% [112/324] vs 23% [36/157], respectively; P =.002). Overall, a higher proportion of nonnucleoside reverse transcriptase inhibitor (NNRTI)-naive patients had a viral load below 200 copies/mL compared with NNRTI-experienced patients (43% [115/270] vs 16% [33/211], respectively; P<.001). Baseline **HIV-1** **hypersusceptibility** to efavirenz (< or = 0.4-fold difference in susceptibility compared with reference virus) was associated with suppression of viral load at 24 weeks to below 200 copies/mL (odds ratio [OR], 3.49; 95% confidence interval [CI], 1.62-7.33; P =.001), and more than 10-fold reduction in efavirenz susceptibility, with less likelihood of suppression at 24 weeks (OR, 0.28; 95% CI, 0.09-0.87; P =.03). CONCLUSIONS: In this study of antiretroviral-experienced patients with advanced immunodeficiency, viral load suppression to below 200 copies/mL was achieved in 31% of patients with regimens containing 4 or 5 new drugs. Use of 2 PIs, being naive to NNRTIs, and baseline **hypersusceptibility** to efavirenz were associated with a favorable outcome.

L25 ANSWER 19 OF 27 MEDLINE on STN

2002103031. PubMed ID: 11832698. Role of sequencing in therapy selection. Keiser P. (Department of Medicine, University of Texas Southwestern Medical Center at Dallas, USA.) Journal of acquired immune deficiency syndromes (1999), (2002 Feb 1) Vol. 29 Suppl 1, pp. S19-27. Ref: 30. Journal code: 100892005. ISSN: 1525-4135. Pub. country: United States. Language: English.

AB The goals of sequencing antiretroviral agents are to preserve as many treatment options as possible, minimize drug toxicity, and prolong

suppression of HIV. There are numerous options for sequencing antiretroviral agents when treatment fails. The most common reasons for treatment failure are the emergence of resistance and poor adherence. Data indicate that **protease** inhibitor therapy enhanced by ritonavir may delay the development of resistance longer than nonboosted **protease** inhibitor therapy. The results of using efavirenz or abacavir to simplify **protease** inhibitor treatment regimens for **HIV**-suppressed patients are promising. Although resistance to nonnucleoside reverse transcriptase inhibitors is a serious problem, sequencing them after zidovudine or abacavir therapy may be effective because of the **hypersusceptibility** to nonnucleoside reverse transcriptase inhibitors exhibited by viral populations in many nucleoside reverse transcriptase inhibitor-experienced patients. New antiretrovirals with greater tolerability, higher genetic barriers, and less cross-resistance than existing agents are needed to achieve further dramatic advances in treating **HIV** infection.

L25 ANSWER 20 OF 27 MEDLINE on STN

2001665578. PubMed ID: 11700580. Human immunodeficiency virus type 1 hypersusceptibility to amprenavir in vitro can be associated with virus load response to treatment in vivo. Zachary K C; Hanna G J; D'Aquila R T. (Infectious Disease Division, Massachusetts General Hospital, Partners AIDS Research Center, and Harvard Medical School, Boston, MA 02114, USA.. kzachary@partners.org) . Clinical infectious diseases : an official publication of the Infectious Diseases Society of America, (2001 Dec 15) Vol. 33, No. 12, pp. 2075-7. Electronic Publication: 2001-11-07. Journal code: 9203213. E-ISSN: 1537-6591. Pub. country: United States. Language: English.

AB The **human immunodeficiency virus** type 1 **protease** mutation N88S, which is occasionally selected by treatment with nelfinavir or indinavir, confers **hypersusceptibility** to amprenavir in vitro. The clinical relevance of this observation is unclear. We report a case of N88S developing after virologic failure of both indinavir- and nelfinavir-containing regimens that was managed successfully with a regimen that contained amprenavir.

L25 ANSWER 21 OF 27 MEDLINE on STN

2001023148. PubMed ID: 10998332. Fitness of human immunodeficiency virus type 1 protease inhibitor-selected single mutants. Martinez-Picado J; Savara A V; Shi L; Sutton L; D'Aquila R T. (Massachusetts General Hospital, Boston, Massachusetts 02129, USA.) Virology, (2000 Sep 30) Vol. 275, No. 2, pp. 318-22. Journal code: 0110674. ISSN: 0042-6822. Pub. country: United States. Language: English.

AB **Human immunodeficiency virus** type 1 (**HIV**-1) evolution under chemotherapeutic selection pressure in vivo involves a complex interplay between an increasing magnitude of drug resistance and changes in viral replicative capacity. To examine the replicative fitness of **HIV**-1 mutants with single, drug-selected substitutions in **protease** (**PR**), we constructed virus that contained the most common mutations in indinavir-selected clinical isolates, **PR** M46I and V82T, and the most common polymorphic change in drug-naive patients, **PR** L63P. These mutants were competed in vitro in the absence of drug against the otherwise isogenic WT virus (NL4-3). Phenotypic drug susceptibility was determined with a recombinant virus assay using a single cycle of virus growth. **PR** M46I and L63P were as fit as WT. However, **PR** V82T was out-competed by WT. None of these mutants had appreciable phenotypic resistance to any of the **protease** inhibitors, including indinavir. The PRV82T mutant was **hypersusceptible** to saquinavir. Thus, the impaired fitness of the V82T single mutant is consistent with its low frequency in **protease** inhibitor-naive patients. The similar fitness of WT (NL4-3), L63P, and M46I is consistent with the common occurrence of L63P in the absence of **protease** inhibitor-selection pressure, but not with the rare detection of M46I in drug-naive patients.

L25 ANSWER 22 OF 27 MEDLINE on STN

2001020733. PubMed ID: 10881368. Reversal of cachexia in patients treated with potent antiretroviral therapy. Scevola D; Di Matteo A; Uberti F; Minoia G; Poletti F; Faga A. (Institute of Infectious Diseases, IRCCS Policlinico S. Matteo, University of Pavia, Italy.) The AIDS reader, (2000 Jun) Vol. 10, No. 6, pp. 365-9, 371-5. Ref: 86. Journal code: 9206753. ISSN: 1053-0894. Pub. country: United States. Language: English.

AB The introduction of HAART has changed the nutritional status of **HIV** patients. In the pre-**protease** inhibitor (PI) era, more than 60% of **HIV**-positive persons presented with protein energy malnutrition (PEM) and vitamin and mineral deficit. This caused progressive physical-metabolic wasting (wasting syndrome/cachexia) and **increased susceptibility** to opportunistic infections and drug toxicity. PEM was a concurrent cause in 80% of deaths attributed to AIDS. Since 1996, the year in which PIs were introduced, the number of patients dying as a result of AIDS has decreased by two thirds, and cachexia is no longer the AIDS terminal phase in developed countries. But different patterns of nutritional status changes have appeared in association with the use of newer anti-**HIV** therapies and with longer survival of **HIV**-infected

patients. A new clinical and laboratory syndrome--lipodystrophy syndrome--now affects patients receiving PI-based therapy. This syndrome consists of changes in body shape that are caused by an abnormal redistribution of fat. Fat accumulates in the abdominal area (truncal and visceral obesity), in the axillary pads (bilateral symmetric lipomatosis), and in the dorsocervical pads ("buffalo hump," "bull neck") but decreases in the legs, arms, and nasolabial and cheek pads (peripheral lipodystrophy). Hyperlipidemia and insulin resistance are also frequently present (metabolic syndrome X). Pathogenic mechanisms of lipid and fat tissue disturbances are discussed in this article, and the clinical approach to patient management and therapeutic options for lipodystrophy and lipid dysmetabolism is evaluated.

L25 ANSWER 23 OF 27 MEDLINE on STN

2000387887. PubMed ID: 10888624. Cathepsin G, a neutrophil-derived serine protease, increases susceptibility of macrophages to acute human immunodeficiency virus type 1 infection. Moriuchi H; Moriuchi M; Fauci A S. (Laboratory of Immunoregulation, National Institute of Allergy and Infectious Diseases, National Institutes of Health, Bethesda, Maryland 20892, USA.. hiromori@net.nagasaki-u.ac.jp) . Journal of virology, (2000 Aug) Vol. 74, No. 15, pp. 6849-55. Journal code: 0113724. ISSN: 0022-538X. Pub. country: United States. Language: English.

AB Neutrophils dominate acute inflammatory responses that generally evolve into chronic inflammatory reactions mediated by monocyte/macrophages and lymphocytes. The latter cell types also serve as major targets for **human immunodeficiency virus** type 1 (**HIV**-1). In this study we have investigated the role of neutrophil products, particularly cathepsin G, in **HIV** infection. Cathepsin G induced chemotaxis and production of proinflammatory cytokines by macrophages but not CD4(+) T cells. Pretreatment with cathepsin G markedly **increased susceptibility** of macrophages but not CD4(+) T cells to acute **HIV**-1 infection. When macrophages were exposed to pertussis toxin prior to cathepsin G treatment, the cathepsin G-mediated effect was almost abrogated, suggesting that enhancement of **HIV**-1 replication by cathepsin G requires Gi protein-mediated signal transduction. Although prolonged exposure to cathepsin G suppressed **HIV** infection of macrophages, serine **protease** inhibitors, which are exuded from the bloodstream later during inflammatory processes, neutralized the inhibitory effect. Neutrophil extracts or supernatants from neutrophil cultures, which contain cathepsin G, had effects similar to purified cathepsin G. Thus, cathepsin G, and possibly other neutrophil-derived serine proteases, may have multiple activities in **HIV**-1 infection of macrophages, including chemoattraction of monocyte/macrophages (**HIV**-1 targets) to inflamed tissue, activation of target cells, and increase in their susceptibility to acute **HIV**-1 infection.

L25 ANSWER 24 OF 27 MEDLINE on STN

1999217706. PubMed ID: 10203056. Restoration of the immune system with anti-retroviral therapy. Autran B; Carcelaint G; Li T S; Gorochov G; Blanc C; Renaud M; Durali M; Mathez D; Calvez V; Leibowitch J; Katlama C; Debre P. (Laboratoire d'Immunologie Cellulaire et Tissulaire, UMR CNRS 7627, Hopital Pitie-Salpetriere, Paris, France.) Immunology letters, (1999 Mar) Vol. 66, No. 1-3, pp. 207-11. Ref: 9. Journal code: 7910006. ISSN: 0165-2478. Pub. country: Netherlands. Language: English.

AB Clinical benefits of highly active anti-retroviral treatments (HAART) are increasingly evidenced by resolving opportunistic infections and malignancies, as well as declining hospitalization and mortality rates [1]. This suggests that potent and sustained suppression of viral replication, at least to some extent, is associated with reconstitution of the immune system even in adult patients treated at advanced stages of the disease. **Increased susceptibility** to opportunistic infections and tumors mainly results from the loss of memory CD4+ T cell reactivity against recall antigens which is an early event in **HIV** disease progression. Primary responses of naive CD4+ T cells against new pathogens are suppressed even earlier in the course of **HIV** disease, and the progressive depletion in naive CD4+ T cells reflects profound alterations in T cell regeneration capacities. Previous studies revealed that monotherapy with ritonavir, a **protease** inhibitor, resulted in a slight improvement in memory CD4+ T cell responses to recall Ags only when detectable prior to onset of therapy, suggesting that the loss of CD4+ T cell reactivity might be irreversible at advanced stages of the disease [2]. In contrast our group demonstrated more recently that restoration in CD4+ T cell reactivity to specific antigens was feasible when HAART was administered in progressors [3]. Here we address some of the questions raised by immune restoration with HAART when administered at advanced stages of the disease.

L25 ANSWER 25 OF 27 MEDLINE on STN

97385705. PubMed ID: 9241719. A 1990s perspective of hepatitis C, human immunodeficiency virus, and tuberculosis infections in dialysis patients. Murthy B V; Pereira B J. (Tufts University School of Medicine, Boston, MA, USA.) Seminars in nephrology, (1997 Jul) Vol. 17, No. 4, pp. 346-63. Ref: 185. Journal code: 8110298. ISSN: 0270-9295. Pub. country: United

Status: Language: English.

AB Chronic infections contribute significantly to morbidity and mortality in dialysis patients. These infections are acquired either before or after initiation of dialysis, and the latter may be via nosocomial modes of transmission. Consequently, policies that deal with infection control in dialysis units have assumed increasing importance. The incidence and prevalence of hepatitis C virus (HCV) infection among patients on dialysis is steadily declining. Nonetheless, the 0.4% to 15% incidence of anti-HCV in hemodialysis (HD) units continues to be a cause for concern. Although nosocomial transmission of HCV infection in HD units has been demonstrated, the Centers for Disease Control and Prevention (CDC), Atlanta, GA, does not recommend dedicated machines, patient isolation, or a ban on reuse in HD patients with HCV infection. Conventional cleansing and sterilization procedures for reprocessing the dialyzers appear to be adequate to inactivate the virus. Over the years, there has been a steady increase in the number of **human immunodeficiency virus** (**HIV**)-infected patients entering end-stage renal disease (ESRD) programs. Transmission of **HIV** infection is extremely unlikely in dialysis units that conform to the standard practice guidelines. Dedicated machines or isolation from other patients are not recommended for patients with **HIV** infection. Risk of acquiring **HIV** infection after an occupational exposure is approximately 0.32%. Nonetheless, a combination of zidovudine and lamuvidine for most parenteral exposures, and the addition of a **protease** inhibitor in high-risk exposures, is recommended. The wide range of immunological derangements in chronic renal failure have been postulated to be the cause for the **increased susceptibility** of dialysis patients to tuberculosis (TB). The high incidence of extrapulmonary disease may be a significant factor in the delay in diagnosis of TB in these patients. In view of their high-risk for exposure to TB, the purified protein derivative (PPD) skin test is recommended on an annual basis in the staff of dialysis units.

L25 ANSWER 26 OF 27 MEDLINE on STN

97343410. PubMed ID: 9199966. HIV type 1 Tat protein enhances activation-but not Fas (CD95)-induced peripheral blood T cell apoptosis in healthy individuals. Katsikis P D; Garcia-Ojeda M E; Torres-Roca J F; Greenwald D R; Herzenberg L A; Herzenberg L A. (Department of Genetics, Stanford University School of Medicine, CA 94305, USA.) International immunology, (1997 Jun) Vol. 9, No. 6, pp. 835-41. Journal code: 8916182. ISSN: 0953-8178. Pub. country: ENGLAND: United Kingdom. Language: English.

AB T cell apoptosis may play an important role in the depletion and functional defects of T cells in **HIV** disease. A number of investigators have shown that peripheral blood T cells in **HIV** disease undergo spontaneous and activation-induced apoptosis. We found recently that peripheral blood T cells from **HIV**+ individuals undergo apoptosis when stimulated through Fas. Also, a number of investigators have shown that Tat protein from **HIV**-1 can increase spontaneous and activation-induced apoptosis. In the present study we examined the effect of **HIV** type 1 Tat protein on spontaneous, activation-induced and Fas-induced apoptosis of peripheral blood T cells from **HIV**- individuals. We find that Tat protein has no effect on spontaneous apoptosis but does enhance activation-induced apoptosis of both CD4+ and CD8+ T cells. Tat, however, failed to enhance Fas-induced apoptosis of CD4+ and CD8+ T cells. Examining the mechanisms by which Tat induces apoptosis, we found that inhibitors of reactive oxygen intermediate (ROI) generation or neutralizers of ROI, such as rotenone, a potent inhibitor of mitochondrial complex I of the respiratory chain, and 3,3,5,5-tetramethylpyrroline N-oxide (TMPO), an electron spin trap, could both enhance the spontaneous apoptosis induced by Tat. This enhancement of Tat-induced apoptosis by rotenone and TMPO was independent of ICE activation as it could not be inhibited by the tripeptide z-VAD-fmk, an irreversible inhibitor of ICE/ced-3 **protease** homologs. These findings suggest that Tat induced enhancement of activation-induced cell death may involve complex mechanisms, some of which are ROI independent. These results indicate that a **HIV**-specific mechanism other than Tat is responsible for the previously observed **increased susceptibility** of peripheral blood T cells from **HIV**-infected individuals to undergo apoptosis in response to Fas stimulation.

L25 ANSWER 27 OF 27 MEDLINE on STN

96100736. PubMed ID: 7486905. Cross-resistance analysis of human immunodeficiency virus type 1 variants individually selected for resistance to five different protease inhibitors. Tisdale M; Myers R E; Maschera B; Parry N R; Oliver N M; Blair E D. (Wellcome Research Laboratories, Beckenham, Kent, United Kingdom.) Antimicrobial agents and chemotherapy, (1995 Aug) Vol. 39, No. 8, pp. 1704-10. Journal code: 0315061. ISSN: 0066-4804. Pub. country: United States. Language: English.

AB **Human immunodeficiency virus** type 1 (**HIV**-1) **protease** inhibitor-resistant variants, isolated on passage of **HIV**-1HXB2 in MT-4 cells with five different **protease** inhibitors, have been examined for cross-resistance to five inhibitors. The **protease** inhibitors studied were Ro 31-8959, A-77003, XM323, L-735,524, and VX-478. Resistant variants with two to four mutations within their **protease** sequence and

inhibitors within six to eight passes in cell culture. Passage of a zidovudine-resistant mutant in Ro 31-8959 generated a dual reverse transcriptase- and **protease**-resistant virus. Variants were cloned directly into a modified pHXB2-D infectious clone for cross-resistance analysis. Although the resistant variants selected possessed different combinations of **protease** mutations for each inhibitor, many showed cross-resistance to the other inhibitors, and one showed cross-resistance to all five inhibitors. Interestingly, some mutants showed **increased susceptibility** to some inhibitors. Further **HIV** passage studies in the combined presence of two **protease** inhibitors demonstrated that in vitro it was possible to delay significantly selection of mutations producing resistance to one or both inhibitors. These studies indicate that there may be some rationale for combining different **protease** inhibitors as well as **protease** and reverse transcriptase inhibitors in **HIV** combination therapy.

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L3 11 S L2 AND (HIV?/CLM OR HUMAN IMMUNODEFICIENCY VIRUS/CLM)
L4 3 S L3 AND (HYPERSUSCEPTIBIL?/CLM OR INCREASE? SUSCEPTIBILIT?/CLM
L5 8918 S (HIV?/CLM OR HUMAN IMMUNODEFICIENCY VIRUS/CLM)
L6 1383 S L5 AND (PR/CLM OR PROTEASE/CLM)
L7 3 S L6 AND (HYPERSUSCEPTIB?/CLM OR INCREASE? SUSCEPTIBIL?/CLM)
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L13 25650 S (HIV OR HUMAN IMMUNODEFICIENCY VIRUS)
L14 2534 S L13 AND (PR OR PROTEASE)
L15 3 S L14 AND (HYPERSUSCEPTIB? OR INCREASE? SUSCEPTIB?)
L16 0 S L15 NOT L12

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L19 22 S L18 AND (PR/AB OR PROTEASE/AB)
L20 21 S L19 AND (HIV?/AB OR HUMAN IMMUNODEFICIENCY VIRUS/AB)
L21 6 S L20 AND (HYPERSUSCEPTIB?/AB OR INCREASED SUSCEPTIB?/AB)
L22 122819 S (HIV?/AB OR HUMAN IMMUNODEFICIENCY VIRUS/AB)
L23 7120 S L22 AND (PR/AB OR PROTEASE/AB)
L24 33 S L23 AND (HYPERSUSCEPTI?/AB OR INCREASED SUSCEPTIBIL?/AB)
L25 27 S L24 NOT L21

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ALL L# QUERIES AND ANSWER SETS ARE DELETED AT LOGOFF
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STN INTERNATIONAL LOGOFF AT 13:21:45 ON 23 JUL 2007